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SEASONAL VARIATION IN WATER CONTENT AND IN TRANSPIRATION OF LEAVES OF FAGUS AMERICANA, HAMAMELIS VIRGINIANA, AND QUERCUS ALBA.

BY

Arabel W. Clark, Ph. D.
With Chart Figures I—XXXIII.

(Thesis presented to the Faculty of the Graduate School in partial fulfilment of the requirements for the Degree of Doctor of Philosophy)

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I. The Problem

The problem is, to determine the water content and transpiration in the leaves of *Fagus americana*, *Hamamelis virginiana*, and *Quercus alba* during successive seasonal changes. *Fagus americana*, *Hamamelis virginiana*, and *Quercus alba* are all native species of our Pennsylvania hardwoods, and in this connection, have been studied, as far as possible, under normal conditions in their natural habitat.

By "water content" is meant, the percentage of the whole fresh leaf that is water; it includes both the water within the walls of the leaf cells and that held in the intercellular spaces. "Transpiration" is used in the sense of Burgerstein[1] to mean the amount of moisture given off in the vapor form from the surface of the leaf; it includes both epidermal and stomatal evaporation. The work started with the earliest leaf development in the Spring of 1915, extended through the Summer, and ended with the leaf-fall in the Autumn months.

II. Work Previously Done

Much work has been done along the lines of transpiration. It has been studied extensively from morphological, physiological, ecological, and purely physical standpoints, and detailed research has been carried on showing the relation between transpiration and sap flow. While Münsch[2] has shown that water content in plants is a determining factor in the appearance of fungous diseases, little connected work has ever been done heretofore along the lines of transpiration and the water content of leaves.

III. Available Methods

Transpiration

As pointed out by Burgerstein[3] any of the following methods are available for determining the amount of water lost in transpiration:

a. Physical methods.
   1. direct weighing. This consists of weighing specimen and apparatus both before and after a certain transpiration period.
   2. collecting and weighing water vapor in a closed room.

---

[1] *Burgerstein*, "Die Transpiration der Pflanzen," 1904, p. 3.

[2] *Münsch*, "Untersuchungen über Immunität und Krankheitempfänglichkeit der Holzpflanzen." Naturwiss. Zeitschr. f. Forst. u. Landw. 1909, 7:54-75, 87-114, 129-160.

[3] *Burgerstein*, "Transpiration der Pflanzen," 1904, p. 4-28.

    3. self-registering apparatus made especially to determine amount of transpiration.
    4. calculating the amount of water absorbed by plants and considering this the equivalent of the amount transpired.

- b. Chemical methods.
    1. determination of increase of water absorbed by such substances as calcium chloride and concentrated sulphuric acid.
    2. color change in paper impregnated with palladium chloride and cobalt chloride.

The method used was that of direct weighing, which, according to Burgerstein is the most reliable.

Water Content

For determining water content the following methods are offered:

- a. Physical methods. Any physical property which changes with the water content offers a possible basis for experimentation.
    1. electrical properties, for example, electrical resistance.
    2. thermal properties, such as thermal conductivity and capacity for heat.
    3. mechanical properties, such as weight and tensile strength.
    4. optical properties, such as coefficient of absorption and reflection of radiation. It is known, for instance, that water very strongly absorbs infra-red radiation. The amount of absorption would probably indicate the amount of water present in the leaf.
- b. Chemical methods.
    1. qualitative test for water. This test sensitive to less than 0.1 mg. is easily and quickly made by bringing the substance to be tested into contact with calcium carbide in the presence of an acetylene solvent. This is then decanted or distilled into an ammoniacal solution of cuprous chloride.[4]

The method selected was the one which had been satisfactorily used in preliminary experiments carried on in the summer of 1914 at the University of Michigan Biological Station. It was based upon the mechanical property of weight.

---

[4] Journal Franklin Institute, March, 1916. p. 408.

IV. PRELIMINARY PROBLEM

Location

The Michigan Biological Station is situated on the south side of Douglas Lake, about twenty miles south of Mackinac Island, in the northern part of the Southern Peninsula of Michigan. Immediately surrounding the camp is a xerophytic association of aspens; a mile and a half east of camp lies a bog, in which are found the same species of aspen in hydrophytic habitat.

The Problem

The problem in this preliminary work was to determine the water content in the leaves of *Populus tremuloides* and *Populus grandidentata* under both typically xerophytic and hydrophytic conditions for both clear and cloudy weather. The topic thus naturally resolved itself into the following heads:

1. Populus tremuloides = xerophytic = clear
2. Populus tremuloides = xerophytic = cloudy.
3. Populus tremuloides = hydrophytic = clear.
4. Populus tremuloides = hydrophytic = cloudy.
5. Populus grandidentata = xerophytic = clear.
6. Populus grandidentata = xerophytic = cloudy.
7. Populus grandidentata = hydrophytic = clear.
8. Populus grandidentata = hydrophytic = cloudy.

Apparatus

The apparatus used was in all cases simple and unoriginal. It consisted of 144 homeopathic vials with paper labels and tight-fitting corks; chemical balances, accurate to .5 of 1%; a gas oven; a Clements' photometer, loaded with Solio paper; and an egg-beater psychrometer, fitted with wet and dry bulb Fahrenheit thermometers.

Detailed Method

For purposes of curtailing the work, the first and fifth, second and sixth, third and seventh, and fourth and eighth divisions of the problem were carried through simultaneously. The work thus resolved itself into four different experiments, readings for which were taken every hour for 24 consecutive hours. As far as possible, leaves of apparently the same size were selected from the same trees. Experiments for similar conditions were not duplicated and because of weather changes, readings for xerophytic cloudy conditions were got for 12 hours only.

The three series of 48 bottles, labelled and tightly corked, were marked in ink on both label and cork: *1t*, *1g*, *2t*, *2g*, etc., and placed in order in a shallow box of convenient size. The number in each case stood for the reading of the experiment, and the letter indicated the species of leaf, whether *tremuloides* or *grandidentata*. This system of labelling not only indicated the species of leaf, but proved itself most valuable in associating any cork always with its proper bottle, a device to reduce the number of weighings. Each bottle with cork was then weighed accurately, registered according to label, and arranged in series according to number. Three such series were kept in readiness, each designated by a special mark (check, comma, cross) to avoid any possible cork or bottle confusion.

When the experiment was started, the first reading was taken by removing three to six leaves (depending upon size) from *Populus tremuloides*, rolling them quickly, and corking them tightly in the bottle marked *1t*. Immediately the psychrometer was placed vertically with its bulbs in the position formerly held by the leaves pulled off. The wet and dry readings were tabulated. The photometer was placed in a similar position and exposed to the light. The number of seconds required for suitable coloration was recorded together with the number of the disk exposed. A similar photometric reading was taken immediately in the open, and the whole process repeated for *Populus grandidentata*.

After the readings were completed, the corked bottles with their contained leaf rolls were weighed. The object of the tight cork was to prevent the escape of evaporated water before weighing. All results were tabulated and the corks were then removed and arranged in series for future convenience. The 48 corkless bottles with their leafy contents were placed in shallow boxes and put in the gas oven. In order to prevent carbonization of the leaf tissues, the oven was regulated so as never to exceed 100° C. They were kept in the oven until there was no loss in weight, and this required on the average about 24 hours. Upon removal from the oven, the bottles were again fitted with their respective corks, and another weighing was taken, this time of bottle, cork, and dried leaves within.

The following data were thus obtained:

W1 = weight of labelled bottle + labelled cork
W2 = weight of labelled bottle + labelled cork + fresh leaves
W3 = weight of labelled bottle + labelled cork + dried leaves

$W2 - W1$ = weight of fresh leaves

$W3 - W1$ = weight of dried leaves

$(W2 - W1) - (W3 - W1)$ = weight of water in fresh leaves

$W2 - W3$ = weight of water in fresh leaves

$$\frac{W2 - W3}{W2 - W1} \times 100 = \text{water content, based on weight of the fresh leaves.}$$

For every hour of the 24 consecutive hours, water content for both *Populus tremuloides* and *Populus grandidentata*, based on the weight of the fresh leaves, was reckoned.

Due to lack of anemometers at the Station, only general observations were made as to air movement; no data were got for air pressure; measurements of water content of soil were eliminated; and general weather conditions were roughly summed up as cloudy, clear, rain, etc. Percentages of relative humidity were obtained from the "Marvin Psychometric Tables." Percentages of light intensity were calculated according to the following formula:

$$\frac{ay}{bx} = \text{light intensity, where}$$

x = number of seconds required to produce a good tint on the Solio paper in the shade to be tested.

y = number of seconds required to produce a good tint in the open at the same time.

a = number of seconds required to produce on the standard a tint to match x.

b = number of seconds required to produce on the standard a tint to match y.

The standard was made by exposing the Solio strip for 2, 4, 6, 8, etc., seconds at noon on a bright, sunny day, July 4. All Solio strips were kept carefully in a dark, dry place and were later developed in a solution consisting of a teaspoonful of Hypo dissolved in 4 ounces of water.

Since these figures in light intensity show only the percentage of all available light that is received at each particular reading, the results are practically valueless so far as daily or seasonal consecutive work in water content and transpiration values are concerned.

Data and Results

From readings of the various phases of the experiments, the following tables were compiled:

TABLE I
WATER CONTENT (See Fig. I)

	Conditions		24 hours					8 A. M.—5 P. M.					8 P. M.—5 A. M.				
			Maximum	Time	Minimum	Time	Average	Maximum	Time	Minimum	Time	Average	Maximum	Time	Minimum	Time	Average
July 16 P.t.	Xer.	Clear	75	P. M. 10:30	48:2	A. M. 1:30	59.9	60.7	A. M. 8:30 P. M. 5:15	54.5	P. M. 12:15	59.5	62.3	A. M. 5:30	48.2	A. M. 1:30	60.1
Aug. 17 P.t.	Xer.	Cloudy						58.6	A. M. 8	55.6	P. M. 4	57.2					
Aug. 6 P.t.	Hyd.	Clear	69.4	A. M. 9	60.8	P. M. 8	65.4	69.4	A. M. 9	62.6	P. M. 1	65.4	67.3	Midnt. 12	61.9	A. M. 3	65.2
July 30 P.t.	Hyd.	Cloudy	69.6	A. M. 8:30 P. M. 10:30	61	P. M. 7 P. M.	64.8	69.6	A. M. 8:30	64.1	P. M. 2:30	66.4	64.1	A. M. 4 P. M. 10:30	62	A. M. 1	62.9
July 16 P.g.	Xer.	Clear	61.4	A. M. 1:30	54.2	P. M. 8:30	58.7	59.7	A. M. 10:15	57	A. M. 11:15	58.2	61.4	A. M. 1:30	54.2	P. M. 8:30	59.2
Aug. 17 P.g.	Xer.	Cloudy						60.2	A. M. 9	52.6	A. M. 10	58.4					
Aug. 6 P.g.	Hyd.	Clear	65.6	Midnt. 12	54.5	P. M. 4	60.1	61.6	A. M. 10	54.5	P. M. 4	58.6	65.6	Midnt. 12	56.6	P. M. 10	61.7
July 30 P.g.	Hyd.	Cloudy	63.7	A. M. 6	55.8	P. M. 9	61.1	63.2	A. M. 8:30 P. M. 12:30	56.7	P. M. 4:30	60.8	63	A. M. 4	55.8	P. M. 9	60.9

TABLE II

Temperature (Fahrenheit) (See Fig. II)

	Conditions		8 P. M.—5 A. M.					8 A. M.—5 P. M.					24 hours			
			Maximum	Time	Minimum	Time	Not taken	Maximum	Time	Minimum	Time	Not taken	Maximum	Time	Minimum	Time
July 16 P.t.	Xer.	Clear	86	P. M. 7:30	49	P. M. 2:15		62	A. M. 8:30	49	P. M. 2:15		81	P. M. 10:30	56	A. M. 3:30
Aug. 17 P.t,	Xer.	Cloudy						100	A. M. 11	47	P. M. 3					
Aug. 6 P.t.	Hyd.	Clear	100	A. M. 3	44	Noon 12		75	A. M. 8	44	Noon 12		100	A. M. 3	79	P. M. 9
July 30 P.t.	Hyd.	Cloudy	94	A. M. 2	51	P. M. 4:30		80	P. M. 12:30	51	P. M. 4:30		94	A. M. 2	84	P. M. 8
July 16 P.g.	Xer.	Clear	78	P. M. 11:30	47	P. M. 2:15		63	P. M. 4:15	47	P. M. 2:15		78	P. M. 11:30	60	A. M. 2:30 A. M. 3:30
Aug. 17 P.g.	Xer.	Cloudy						100	A. M. 11	56	P. M. 2					
Aug. 6 P.g.	Hyd.	Clear	100	A. M. 3	44	Noon 12		75	A. M. 8	44	Noon 12		100	A. M. 3	79	P. M. 9
July 30 P.g.	Hyd.	Cloudy	94	A. M. 2	51	P. M. 4:30		80	P. M. 12:30	51	P. M. 4:30		94	A. M. 2	84	P. M. 8

TABLE III
Relative Humidity (See Fig. II)

	Conditions		24 hrs.				8 A. M.—5 P. M.				8 P. M.—5 A. M.			
			Maximum	Time	Minimum	Time	Maximum	Time	Minimum	Time	Maximum	Time	Minimum	Time
July 16 P.t.	Xer.	Clear	86	P. M. 7:30	49	P. M. 2:15	62	A. M. 8:30	49	P. M. 2:15	81	P. M. 10:30	56	A. M. 3:30
Aug. 17 P.t.	Xer.	Cloudy					100	A. M. 11	47	P. M. 3				
Aug. 6 P.t.	Hyd.	Clear	100	A. M. 3	44	Noon 12	75	A. M. 8	44	Noon 12	100	A. M. 3	79	P. M. 9
July 30 P.t.	Hyd.	Cloudy	94	A. M. 2	51	P. M. 4:30	80	P. M. 12:30	51	P. M. 4:30	94	A. M. 2	84	P. M. 8
July 16 P.g.	Xer.	Clear	78	P. M. 11:30	47	P. M. 2:15	63	P. M. 4:15	47	P. M. 2:15	78	P. M. 11:30	60	A. M. 2:30 A. M. 3:30
Aug. 17 P.g.	Xer.	Cloudy					100	A. M. 11	56	P. M. 2				
Aug. 6 P.g.	Hyd.	Clear	100	A. M. 3	44	Noon 12	75	A. M. 8	44	Noon 12	100	A. M. 3	79	P. M. 9
July 30 P.g.	Hyd.	Cloudy	94	A. M. 2	51	P. M. 4:30	80	P. M. 12:30	51	P. M. 4:30	94	A. M. 2	84	P. M. 8

TABLE IV (Results)

Conditions	Results	Proportion	Difference (%)
Xer. clear	P.t.>P.g.	20:4	1.2
Xer. cloudy	P.g.>P.t.	9:0	1.2
hyd. clear	P.t.>P.g.	24:1	5.3
hyd. cloudy	P.t.>P.g.	24:1	3.7
Species			
P.t. (day)	Xer. clear>xer. cloudy	8:1	2.3
P.t. (night)	Hyd. clear>hyd. cloudy	7:2	2.2
P.t. (day)	Hyd. cloudy>hyd. clear	10:5	.4
P.t.	Hyd. clear>xer. clear	23:1	5.5
P.t.	Hyd. cloudy>xer. cloudy	9:0	9.3
P.g. (day)	Xer. cloudy>xer. clear	6:3	.2
P.g. (night)	Hyd. clear>hyd. cloudy	6:2	1.1
P.g. (day)	Hyd. cloudy>hyd. clear	14:2	2.1
P.g.	Hyd. clear>xer. clear	17:5	1.4
P.g.	Hyd. cloudy>xer. cloudy	9:0	2.5

It is seen from Table I that there is no definite time for maxima and minima water contents. Fig. I shows however that, as a general rule, in spite of numerous fluctuations, there is a gradual increase in water content by night, and a gradual decrease by day. It shows likewise that the curves of the two species of Populus, when compared under similar conditions of habitat and atmosphere, follow each other rather closely. This fact indicates the influence of a common factor. By comparing Tables I, II, III and Figures I, II, it is evident that water content is independent of both temperature and relative humidity.

For both *Populus tremuloides* and *Populus grandidentata* under similar atmospheric conditions, water content is greater under hydrophytic than under xerophytic habitat. Under xerophytic cloudy conditions, day readings only were obtained, and with this one exception, water content for *Populus tremuloides* was found to be greater than for *Populus grandidentata*. Day readings under both xerophytic and hydrophytic situations were found for both species to be greater under clear than under cloudy conditions. One exception to this was for *Populus tremuloides* under xerophytic conditions. By night for both species, under hydrophytic situations, water content for clear was greater than for cloudy conditions. Irregularities in general results occur only in *P. tremuloides* under xerophytic conditions, and might probably be accounted for in scarcity of moisture available in the dry soil.

Conclusions

From above results the following conclusions may be drawn:

1. Average water content for *Populus tremuloides* is 61.8%.
2. Average water content for *Populus grandidentata* is 59.6%.
3. There is no definite period for maxima and minima water content.
4. With fluctuations, there is a general increase in water content by night, and a general decrease by day.
5. Under like conditions curves for both species follow each other more or less closely, indicating a common influential factor.
6. Water content is independent of both temperature and relative humidity.
7. Under like atmospheric conditions, for both species, water content under hydrophytic is greater than under xerophytic conditions.
8. With the exception of xerophytic cloudy results, where day readings only were obtained, *Populus tremuloides* water content was greater than that for *Populus grandidentata.*
9. With the exception of *Populus tremuloides* under xerophytic situations by day, water content for cloudy conditions was greater than that for clear.
10. By night for both species, water content for hydrophytic clear was greater than for hydrophytic cloudy conditions.
11. Irregularities are prominent in *Populus tremuloides* under xerophytic conditions.

V. The Present Problem

Location and Ecological Conditions

While the main part of the problem at hand was to determine the water content in leaves of *Fagus americana*, *Hamamelis virginiana*, and *Quercus alba* during the seasonal changes, parallel experiments regarding the amount of transpiration for these leaves were run simultaneously.

The work was done on a Brandywine farm located 6 miles southwest of West Chester, Pa., and 30 miles west of Philadelphia. The experimental field was confined to the northwest corner of a twenty acre woodlot, whose timber had been removed for the first time, 25 years ago in lumbering processes. Here is now a splendid second growth of hardwoods with a few towering relics of the original stand.

The plot selected for the experimental work was irregular in outline: its northern boundary line was a twelve foot cart-road leading into the woods, its southern one was a shallow stream 10 feet in width, fed by a spring 300 feet farther east; its western boundary was a fence, dividing the woods from a broad, open pasture; its eastern border was an imaginary line about 20 feet long running between the cart-road and the stream. The plot thus irregular, had its greatest length of 70 feet, and its greatest width of 30.

During the previous summer (1914) a blighted chestnut tree had been removed from the western end of the plot, leaving a large, solid, two-foot stump. The removal process had resulted in the destruction of most of the secondary growth of the plot, as well as of many young beech saplings. In the plot were found the following species:

(1) Trees:

Fagus americana	15	individuals
Quercus alba	13	”
Cornus florida	7	”
Hamamelis virginiana	7	”
Liriodendron tulipifera	6	”
Castanea dentata	4	”
Acer rubrum	4	”
Prunus serotina	3	”
Fraxinus americana	1	”
Nyssa sylvatica (60 ft.)	1	”

(2) Shrubs (few and scattered:)

Vaccinium stamineum
Rhus toxicodendron
Viburnum acerifolium
Diervilla lonicera

(3) Herbs (few and scattered:)

Lysimachia punctata
Polygonatum biflorum
Impatiens pallida

The forest floor was covered with leaf-mold to a depth of 10-15 inches. During the whole season there was no scarcity of water supply, for not only did the stream run on two sides of the plot, but the season was one of exceptionally great and frequent rainfall, as the following table shows:[5]

---

[5] U. S. Department of Agriculture, Weather Bureau, Monthly Meteorlogical Summary, 1915, Philadelphia, Pa.

TABLE V

Month	Precipitation		No. Clear days
	Total	Normal	
May	4.12	3.20	10
June	3.45	3.30	8
July	5.02	4.33	7
August	6.84	4.61	6
September	0.46	3.38	12
October	1.98	3.10	14

The average level of the forest floor above water line was 3 ft. 2. in.

Variation and Description of Apparatus

As in preliminary experiments, the apparatus used for getting water content was plain and simple. The psychrometer was home-made—constructed from a Dover egg-beater: tin clamps were attached to hold the thermometers firmly in place, and certain unnecessary wires and plates were removed to avoid interference. A spirit-level was in constant use in adjusting the chemical balances and the improvised table on which they rested. The Clements' photometer with Solio strips was again used in light tests The homeopathic vials were replaced by plain, business-sized envelopes. For drying purposes, the warming oven of the kitchen range in the farm-house proved adequate and satisfactory. For transpiration tests three homeopathic vials, vaseline, medicated cotton, a pint tin cup, and shears were provided.

Variation and Description of Methods

A heavy, well-seasoned, 2-inch plank, 2½ feet long by 2 ft. wide, was placed, leveled, and permanently fastened on the above mentioned chestnut stump. On this was placed on end and leveled, a small, heavy dry goods box which sheltered the balances. The box was completely covered with a thick, woolen blanket, over which was spread a pliable oil-cloth. These coverings served to protect the scales from air currents during weighing processes.

The leaves were pulled from the tree, were weighed immediately, and were placed in a series of dated envelopes marked *8B*, *8W*, *8O*, *9B*, 9W *9O*, etc. The label indicated the hour of the day and the kind of leaf—beech, witchhazel, or oak. After each weighing, psychrometric and photometric readings were taken, and general weather conditions noted. Such readings were made hourly from 8 A.M. to 5 P.M. either weekly or semi-seekly from the first of May to the middle of October.

At the end of each day's work, a paste board box containing the various envelopes with their leaves was placed in the warming oven. No constant temperature could be maintained here. A wood fire was made up 3 times daily for getting meals, and often, depending upon household needs, it was kept burning for the greater part of the day. At its hottest, the oven registered 118° F. After a week the leaves were taken from the oven, removed from their respective envelopes, and weighed. From the fresh and dry weights, the water content, based upon the fresh weight, was calculated. (See p.112.)

For transpiration tests, attached twig ends were bent about a foot from the tip into a pint cup filled with water. Since wetting of leaves, according to Haberlandt,[6] Wiesner,[7] and Burgerstein,[8] hastens transpiration rate, great care was taken that the leaves did not come in contact with the water. Following the method of De Vries,[9] the twigs were cut off under water, and then without permitting air tocome in contact with the cut surface, they were transferred to homeopathic vials containing water.

The vials were about three-fourths full of water, and a cork was made by wrapping cotton closely around the stem of the twig and pushing it tightly into the mouth of the bottle. The cotton cork was covered with a thick layer of vaseline to exclude all air, and the vaseline was covered with a thin layer of cotton to prevent the leaves above from coming in contact with the vaseline. Normal transpiration would have been interfered with had the leaf surface received a coat of vaseline.

These experiments were prepared by 7 A. M. and the vials containing the twigs were placed always in the same order in the same shallow wooden box, which was kept always in the same position on top of the oil-cloth, covering the above mentioned dry-goods box. Thus placed, the twigs were left for an hour to overcome the shock of separation from the tree and to become adjusted to new conditions.

[6] *Haberlandt, F.* Das Austrocknen abgeschnittener und benetzter, sowie abgeschnittener und nicht benetzter grüner Blätter und Pflanzenteile. (Wissensch. prakt. Unters, auf dem Gebiete des Pflanzenbaues, herausg. von Fr. Haberlandt, Bd. II, Wien 1877, p. 130.)

[7] *Wiesner.* Studien über das Welken von Blüten und Laubsprossen. Ein Beitrag zur Lehre von der Wasseraufnahme, Saftleitung und Transpiration der Pflanzen. (Ebenda, Bd. LXXXVI, 1882, p. 209.)

[8] *Burgerstein,* Die Transpiration der Pflanzen, 1904, p. 68.

[9] *De Vries.* Über das Welken abgeschnittener Sposse. (Arb. d. Botan. Inst. Wurzburg, Bd. I, Leipzig 1874, p. 287.) Burgerstein. Die Transpiration der Pflanzen, 1904, p. 73.

At eight o'clock the first weighings were made and tabulated. The readings which were taken hourly, consisted of the weight of the bottle, water, cork, and twig. At the end of the day the leaves were removed from their twigs, placed in labelled envelopes, and dried in the warming oven.

W1 = weight of bottle, cork, water, twig, at 8 A. M.
W2 = weight of bottle, cork, water, twig, at 9 A. M.
W3 = weight of dried leaves.
W1-W2 = weight of water lost in transpiration between 8 and 9 A. M.

$$\frac{W1 - W2}{W3} \times 100 = \text{percentage of water lost in transpiration, based on dry leaf weight, between 8-9 A. M.}$$

Thus hourly water loss was calculated for beech, witchhazel, and oak for each day's experiment.

Because the experiments for water content and for transpiration were made at the same time, no separate data for light and atmospheric conditions were got for transpiration. Light intensity percentages proved valueless for successive daily and seasonal work. Slight error in transpiration rate might have crept in for the early hours of the day when the leaves in the morning had been covered with dew. This occurred only once, August 9, and then the dew was carefully absorbed by blotters before the experiments were started.

According to Haberlandt,[10] and Klebahn,[11] water is lost in transpiration by the lenticels. The present method used did not take into consideration the water transpired by stems.

Selection of foliage of the beech was limited in the early summer by the presence of numerous egg-cases of some unknown insect. These egg-cases were found on the ventral side only of the leaf. Oak galls, on the oak leaves, were evident, but not numerous. On the witchhazel were many galls, containing nymphs of *Hormaphis hamamelidis*[12] which were parasitic on the leaves. Within the galls were also unknown larvae, which fed upon the nymphs, and later emerging, fed upon the tissues of the leaf. As a result, the foliage of the witchhazel was greatly damaged, and selection of leaves, in the late summer, was made most difficult.

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[10] *Haberlandt, G.* Beitrage zur Kenntnis der Lenticellen. (Sitzb. d. K. Akad. der Wissensch. in Wien. Bd. LXXII, 1875, p. 175.)

[11] *Klebahn,* Über die Struktur und die Funktionen der Lenticellen, etc. (Ber. d. Deutsch. Botan. Gesellsch. in Berlin, Bd. I, 1883, p. 113.)

[12] *Pergande, Theodore.* Technical Series, Bulletin, number 9, U. S. Bureau of Entomology— 1901.

Data and Results—From readings and calculated results, the following table was compiled:

TABLE VI

	Water Content						Transpiration					Temperature F°					Humidity				
	Maximum	Time	Minimum	Time	Average	Average variation from Average water content	Maximum	Time	Minimum	Time	Average	Maximum	Time	Minimum	Time	Average	Maximum	Time	Minimum	Time	Average
May 2																					
B	86.9	9	75.8	11	81.9	2.63	1.9	3	.5	2	1.1	65	2	52	8	60.5	83	9	51	4	61.6
W	86.7	3	54.7	12	75.8	4.87	2.2	2	.5	9	1.5										
O	83.7	2	65.9	10	70.7	3.22	.9	10	.2	9	.4										
May 9																					
B	75.9	9	72.9	3	73.9	.46	1.8	10	1	5	1.5	71	3	64	8	67.9	61	9	46	11	51.1
W	78.8	12	75.4	5	77.2	.96	3.4	2	1.8	5	2.4										
O	76.4	3	73.8	5	75.4	.63	2	4	.9	9	1.3										
May 23																					
B	61	8	56.2	2	58.8	1.28	1.7	2	.4	10	1.2	73	1 4 5	65	8	70.5	69	1 2	61	12	65.6
W	73.2	5	69.2	2	70.9	.98	1.2	1	.5	9	.9										
O	74.8	10	53.2	2	69.5	3.19	1.3	10	.01	5	.6										
May 31																					
B	61.4	5	56.7	3	59.5	1.23	2.1	1	.5	9	1.3	74	4	60	8	68.3	69	9	48	2	58.2
W	72.7	8	60.9	4	66.1	2.38	1.7	1	.4	5	.8										
O	68.6	5	64	4	65.8	.86	1.6	1	.3	9	1										

June 12																					
B	61.2	8	57.2	4	59.1	.8	1.3	10	.6	5	1	85	4	72	8	80.4	86	8	58	5	64.9
W	68.4	8	58.4	3	64.3	2.44	1.4	1	.7	11	1.1										
O	62.2	9	57.4	1	59.6	1.03	1.1	10	.2	5	.4										
June 20																					
B	76.6	10	54.7	3	60	3.24	2.6	1	.7	9	1.4	80	1 2 3 4 5	73	8	77.8	86	8	61	4	72.4
W	69.2	3	59.2	9	65	2.91	1.6	1	.4	9	.8										
O	64.8	10	61.5	4	63	1.01	1.8	1	.5	5	1										
June 25																					
B	67.5	11	53.3	12	58.8	2.9	1.5	1	.8	4	.1	79	4	64	8	72.7	70	8	52	3	57.3
W	66.6	8	57	10 3	60.9	3.4	.9	1	.5	9 12	.6										
O	68	8	51.7	4	59.9	3.00	2.7	1	.9	9	1.3										
June 28																					
B	64.3	8	53.6	10	58	1.68	2.2	1	.7	3	1.3	78	3 4	63	8	72.6	69	8	49	4	56.4
W	65.7	5	55.7	11	62	2.53	1.2	1	.3	9	.5										
O	76.4	8	57.6	11 3	61.1	2.9	2.5	1	.7	9	1.2										
July 1																					
B	59.6	2	57.4	11	58.3	.61	2.3	1	.8	5	1.4	81	1 3	73	8	78.1	74	9	57	1	67.3
W	66.1	2	58.4	3	61.5	1.92	2.2	1	.7	5	1.2										
O	62.8	9	57.9	3	60.7	1.00	2.8	1	1.2	5	1.6										
July 6																					
B	64.4	4	55.2	5	61.2	1.93	2.4	1	.9	5	1.6	76	3 4	67	8	72.7	65	11 12	44	3 4	54.6
W	63.3	12	58.9	9	61.3	1.42	1.4	10	.2	5	.6										
O	64.2	10	59.3	3	61.1	.96	1.4	10	.1	1	.7										

TABLE VI.—Continued

	Water Content						Transpiration					Temperature Fahrenheit°					Humidity				
	Maximum	Time	Minimum	Time	Average	Average variation from Average water content	Maximum	Time	Minimum	Time	Average	Maximum	Time	Minimum	Time	Average	Maximum	Time	Minimum	Time	Average
July 10																					
B	61.5	11	58.7	5	60.1	1.02	2.5	2	.4	9	1.0	77	2 3	65	8	72.6	85	8	52	2	68.4
W	67.7	4	57.6	2	63.6	2.76	1.4	1	.4	9	.8										
O	62.5	10	59.8	5	61.2	.94	2.9	12	.3	9	.9										
July 14																					
B	62.7	10	57.5	3	60.3	1.36	2.7	1	.8	9	1.7	84	3 4	72	8	79	82	5	56	3	67
W	68.4	9	61	11	64.5	2.7	1.3	1	.3	5	.7										
O	62.9	8	59.1	3	60.4	.76	2.7	1	.4	12	1.4										
July 18																					
B	61.7	10	59.1	4	60.8	.57	3.5	1	1.0	9	2.1	86	3	73	8	80.4	70	9	46	2	62.2
W	67.5	10	58.4	4	62.6	2.34	1.6	1	.7	4	1.0										
O	60.3	11	55.8	12	57.8	1.06	1.9	3	.2	2	.5										

July 22																				
B	61.8	11	52.4	5	58.8	2.24	1.5	1	.4	9	1.0	79	3	66	8	73.1	90	8	57	3	7.11
W	67.1	8	60.2	4	63	1.98	.9	1	.2	9	.6										
O	58.3	8	54.8	5	57.1	.84	1.3	1	.2	9	.7										
July 26																				
B	60.3	10	57	4	58.8	.79	1.8	1	.3	9	1.1	82	3	67	8	76.3	90	8	57	1	70.5
W	64.7	8	49.4	11	59.7	3.71	1.2	1	.2	9	.7										
O	59.8	8	55.3	5	57.1	1.26	1.3	1	.2	9	.6										
July 31																				
B	59.3	9	52.6	10	56.3	2.16	2.7	1	1.1	5	1.7	88	2 3 4	79	8	85	79	8	54	3 4	64.6
W	66.1	11	58.2	8	62.5	1.36	2.6	1	.3	5	1.0										
O	63.8	1	55.6	3	58.5	1.25	1.0	10	.1	5	.5										
Aug. 5																				
B	59.6	3	55.7	2	57.7	1.03	1.3	1	.6	4	.9	75	2	69	8 9	71.9	90	8	72	2	79.5
W	71.2	1	56	11	61.3	3.5	.9	1	.5	9	.7										
O	59.8	8	54.8	5	56.9	1.17	.9	1	.4	9	.6										
Aug. 9																				
B	62.6	5	57.9	4	59.9	1.39	2.2	1	.6	9	1.7	72	1 2 3	68	8	70.3	86	5	61	3	70.3
W	65.1	12	56.8	11	61.1	2.28	.8	1	.2	9	.6										
O	58.7	5	53.2	12	56.5	1.48	1.5	1	.4	5	1.0										
Aug. 13																				
B	60.4	2	52.5	1	56.1	1.79	1.8	11	.5	9	1.2	84	3	75	8	80.8	86	8	53	2 4	65.4
W	65.6	12	55.6	1	59.3	2.47	1.4	1	.3	9	.8										
O	58.4	9	52.1	10	56	1.61	1.2	12	.2	5	.7										

TABLE VI.—Continued

	Water Content						% Transpiration					Temperature Fahrenheit°					% Humidity				
	Maximum	Time	Minimum	Time	Average	Average variation from Average water content	Maximum	Time	Minimum	Time	Average	Maximum	Time	Minimum	Time	Average	Maximum	Time	Minimum	Time	Average
Aug. 16																					
B	62.9	1	54.4	11	58.2	2.93	2.1	12	.9	9	1.6	84	3 4	76	8	80.7	83	5	56	3	66.4
W	65	8	52.7	4	58.6	3.13	1.4	1	.2	5	.7										
O	59.3	9	51.5	4	55.3	1.99	.8	12	.1	5	.7										
Aug. 19																					
B	58	8	52.3	3 4	54.6	1.58	2.3	12	.5	5	1.2	74	1 2 3 4	63	8	70.9	69	8	47	1 2	56.6
W	68.8	4	53.4	2	58.2	3.2	.9	12	.4	9	.7										
O	56.6	1	49.2	9	53.5	1.1	1.3	1	.4	9	.8										
Aug. 23																					
B	72.4	5	58.7	8	60.6	2.31	2.2	12	.4	5	1.3	81	2 3 4	72	8	77.8	77.8	8	57	2 3 4	66.2
W	67.8	3	56.6	10	62.9	2.69	1.1	12	.4	5	.8										
O	61.5	4	51.2	2	57.5	2.55	1.1	10	.4	5	.7										

Aug. 26																					
B	61.1	12	55.8	3	58.6	.91	1.3	12 1	.8	9	1.1	75	2 3 4	66	8	71.7	61	8	51	2	56.5
W	66	2	55.4	9	59.5	2.57	1.4	1	.7	5	1.1										
O	60.3	8	52.1	3	57.1	1.61	1.3	12	.2	5	.7										
Sept. 2																					
B	55.5	1 2	50.9	4	53.7	1.28	2.0	12	.4	5	1.3	71	12 2	61	8	67.8	80	5	51	11	59.5
W	64.1	12	46.8	2	56.3	3.46	.9	11	.2	5	.6										
O	56.4	8	46.9	2	51.3	1.85	.3	11	.1	5	.2										
Sept. 11																					
B	58.6	5	56.2	4	57.7	.62	2.1	1	.4	9	1.5	87	2	71	8	82.2	90	8	50	2	64
W	64.6	3	59.9	2	62	1.14	1.5	1	.3	9	1.1										
O	60	12	53.8	2	56.1	1.28	1.5	1	.3	9	1.0										
Sept. 18																					
B	60.7	5	56.5	12	58.2	1.15	1.4	1	.5	5	.9	75	3	67	8	71.7	86	5	66	3	76.7
W	66.3	9	58.9	1	62.7	1.77	1.0	1	.3	5	.7										
O	58.8	12	49.1	8	55.1	2.02	1.0	1	.2	5	.6										
Sept. 25																					
B	58.8	11	54.4	12	57.2	1.21	1.6	1	.4	9	1.1	73	2	59	8	67.2	83	8	53	2	68.9
W	63.2	12	57.8	2	60.1	1.36	.8	1	.1	9	.5										
O	56.6	1	51.4	2	54.1	1.55	1.3	1	.2	9	.7										
Oct. 3																					
B	59.8	9	56.1	5	58.1	.78	1.7	2	.7	9	1.4	67	2 3	55	8	62.6	70	8	53	2 3	60.1
W	62.9	8 9	57.2	2	60.9	1.58	.9	2	[illegible]	9	.6										
O	57.9	1	50.6	9	54.7	1.67	.9	2	.2	5	.5										

TABLE VI.—Continued

	Water Content						Transpiration					Temperature F°.					Humidity				
	Maximum	Time	Minimum	Time	Average	Average variation from Average water content	Maximum	Time	Minimum	Time	Average	Maximum	Time	Minimum	Time	Average	Maximum	Time	Minimum	Time	Average
Oct. 9																					
B	61.6	1	57.5	11	60	.92	.9	1	.3	9	.7	53	1	46	8	54	67	10	52	1	59.6
W	68.4	8	61.6	11	64.5	1.99	.7	3	.3	9	.5										
O	59.7	12	53.9	3	56.4	1.3	.7	1	.3	9	.5										
Oct. 17																					
B	62.9	11	54.6	1	59.8	1.99	.4	3	.01	9	.3	70	3	58	8	64.7	88	8	59	2 3 4	71.3
W	68.9	12	60.9	3	65.4	2.53	.2	4	.1	9	.1										
O	58.6	9	53.3	2 5	55.5	1.34	.2	3	.1	9	.2										

For daily readings the maxima and minima values of water content occur at no definite periods. As a general rule the two minima for transpiration occur early and late in the day, and the maximum at 1 P. M. The minimum temperature is at 8 A. M. and the maximum at 2-3 P. M. Maximum percentage for relative humidity is at 8 A. M. and minimum at 2-3 P. M. Weather conditions are on the whole regular and constant. While daily variations in transpiration, temperature, and relative humidity are fairly constant, we find no connection between these and water content. Water content therefore must be independent of the influence of transpiration, temperature and relative humidity.

According to Dixon[13] the loss of water due to evaporation "can only be made good by drawing in water from adjacent tracheae, and this pull acting on the upper ends of the cohering columns of sap is propogated downward through the tree. We may then regard secretion or evaporation as the force which actually exerts the tension on the sap, and this tension is transmitted through the leaf cells to the sap in the conducting tracts." Also the same author states that "the amount of transpiration falls off as water is exhausted from the soil." It would seem therefore that so long as the available soil moisture remained constant, there would be no change in the water content of the leaves.

Because of the presence of a stream flowing on two sides of the plot and because of unusually great seasonal rainfall, there could have been no scarcity of available soil moisture, and daily water content should have been constant. Reference to Table VI shows that the average variation from the average water content for each day lies between .4 of 1% for *Fagus americana* (May 9) and 4.87% for *Hamamelis virginiana* (May 2). For the whole season the average variation from the average water content was 1.5% for *Fagus americana*, 2.4% for *Hamamelis virginiana*, and 1.5% for *Quercus alba*. These differences run so low that on the grounds of probable error they may be disregarded. We may consider that from 8 A. M. to 5 P. M. the water content for *Fagus americana*, *Hamamelis virginiana*, and *Quercus alba* is constant.

In the final results of the preliminary experiment it was found (Table IV) that the difference in average water content between the species of *Populus* under various conditions was generally negligible. Under hydrophytic conditions, both cloudy and clear, the water content for *Populus tremuloides* was always greater than for *Populus grandidentata* (5.3% and 3.7% respectively.) Here there is probably the presence of a species difference. The water content for *Populus tremuloides* was always greater under hydrophytic clear and cloudy than under xerophytic clear and cloudy situations (5.3% and 9.3% respectively.) This difference may be accounted for on the grounds that under xerophytic conditions there is probably a scarcity of available soil moisture. The amount of moisture lost in transpiration would exceed that taken in by absorption, and the water content would decrease.

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[13] *Dixon, H.* Transpiration and the Ascent of Sap in Plants, 1914, p. 118-140.

Average transpiration was 1.21%, .83%, and .43% for *Fagus americana, Hamamelis virginiana*, and *Quercus alba* respectively. These results agree with those of von Höhnel,[14] who stated that transpiration in beech leaves was greater than in oak. Rate of transpiration in all three species was highest during the developing season in May, and early June. This confirms Holtermann's[15] work on *Paradenyia.* Wiesner[16] found that falling leaves transpired more rapidly than those that fell later. Fig. XXXIII shows that in *Fagus americana, Hamamelis virginiana*, and *Quercus alba*, on the contrary, transpiration decreases steadily from September 1st to leaf-fall. Water content is in no way influenced by the factors of transpiration, temperature, or relative humidity.

Average water content for the season was 59.9%, 63.1%, and 59.2% for *Fagus americana, Hamamelis virginiana*, and *Quercus alba* respectively. Fig. XXXIII for seasonal variation shows that for *Fagus americana, Hamamelis virginiana*, and *Quercus alba*, during the developing period in May and early June, there is a steady decrease in water content. With fluctuations the water content is constant through June. During July and August there is a slight gradual decrease, and from September 1st to leaf-fall, there is a gradual rise in water content. Benedict[17] finds that in Vitis vulpina L. and certain other plants senile changes are evident. Seasonal variation in water content in *Fagus americana, Hamamelis virginiana*, and *Quercus alba* is probably due to structural changes in the leaves during successive periods of development, maturity, and senility.

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[14] *von Höhnel*, F. v., Üoer das Welken abgeschnittener Sprosse." (Wissensch. prakt. Unters. auf dem Gebiete des Pflanzenbaues, herausg. v. Fr. Haberlandt, Bd. II, Wien 1877, p. 120.)

[15] *Holtermann, K.*, Anatomisch- physiologische Untersuchungen in den Tropen. Die Transpiration der Pflanzen in den Tropen. (Sitzb. d. kgl. preuss. Akad. d. Wissensch. Berlin, Bd. XXX, 1902, p. 656.)

[16] *Wiesner, J.*, Untersuchungen über die herbstliche Entlaubung der Holzgewächse. (Sitzb. d. K. Akad. der Wissensch. Wien, Bd. LXIV, 1871, p. 461.)

[17] *Benedict*, Senile Changes in Leaves of Vitis Vulpina L. and Certain Other Plants. Cornell University Agricultural Experiment Station. 1915. p. 281.

Conclusions

From above results the following conclusions may be drawn:

1) Daily maxima and minima values of water content occur at no definite periods.

2) Daily results for transpiration, temperature, and relative humidity follow more or less definite courses.

3) Water content is independent of the influences of transpiration, temperature, and relative humidity.

4) From 8 A. M. to 5 P. M. water content is constant. This is probably due to the fact that there was no scarcity in available soil-moisture.

5) Average transpiration for the season was 1.21%, .83%, and .43% for *Fagus americana*, *Hamamelis virginiana*, and *Quercus alba* respectively.

6) Rate of transpiration was highest during the early developing-season, and lowest at time of leaf-fall.

7) Average seasonal water content was 59.9%, 63.1%, and 59.2% for *Fagus americana*, *Hamamelis virginiana*, and *Quercus alba* respectively.

8) Water content was highest during the early developing-season, decreased gradually through May and early June, remained more or less constant through middle and late June, decreased slightly through July and August, and increased steadily from September 1st to leaf-fall.

9) Seasonal variations in water content are probably due to seasonal structural changes.

Practical Applications

Münsch's[18] work has shown that the water content is a determining factor in the appearance of fungous diseases in plants. While his work dealt with the water content of the stem, it does not seem improbable that the water content of certain leaves might sometime prove valuable in determining conditions favorable or unfavorable for parasitic fungous growths.

VI. SUMMARY

The object of the present work was to determine seasonal variations in water content and transpiration of leaves of *Fagus americana*, *Hamamelis virginiana*, and *Quercus alba*.

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[18] *Münsch*, 'Untersuchungen über Immunität und Krankheitempfänglichkeit der Holzpflanzen.' Naturwiss Zeitschr. f. Forst. u. Landw. 1909. 7:54-75, 87-114, 129-160.

Results show (1st) that there is no connection between water content and transpiration, temperature, and relative humidity; (2nd) that from 8 A. M. to 5 P. M. there is practically no variation in water content, but that variations are regular and constant for transpiration; (3rd) average water content was greater for *Hamamelis virginiana*, than for *Fagus americana* and *Quercus alba*; (4th) average transpiration was greatest for *Fagus americana* and least for *Quercus alba*; (5th) water content is highest in the Spring, lowers during the Summer, and rises again in the Fall; (6th) transpiration is greatest in the Spring and lowest in the Fall.

A general conclusion of results may be stated as follows:

1) Under the same conditions, water content and transpiration differ in different species.

2) Water content is independent of transpiration, temperature, and relative humidity.

3) Water content is constant from 8 A. M. to 5 P. M. Large supply of soil-moisture may be an influential factor here.

4) Water content varies during seasonal changes. This may be accounted for by structural differences in the leaves from the stages of early development, to those of senility.

VII. Acknowledgments

Great pleasure is taken in expressing to Dr. H. A. Gleason of the University of Michigan, my appreciation of his able and helpful guidance while engaged in the preliminary experiments at the University Biological Station. To Dr. John W. Harshberger of the University of Pennsylvania I owe greatest thanks for his generous and profitable assistance along various lines of the present problem. For construction of simple apparatus and for valuable suggestions I feel deeply indebted to Dr. Enoch Karrer of the Physical Laboratory of the United Gas Improvement Company of Philadelphia. To Mr. and Mrs. William H. Clark of Lenape, Pa. I wish to express my gratitude for the privileges and hospitality of their Brandywine farm during the experimental phases of the subject. My appreciation is also extended to Miss Mabel M. Thackara of the Commercial Course of the Germantown High School, whose valuable manipulation in clerical details has brought the present work to its close.

Philadelphia, Pa. April 6, 1916.

Explanation of Figures

Fig. I.

Water Content of *Populus tremuloides* and *Populus grandidentata.*

(1) xerophytic, clear and cloudy
(2) hydrophytic, clear and cloudy

Fig. II.

Temperature and Relative Humidity for *Populus tremuloides* and *Populus grandidentata.*

(1) xerophytic, clear and cloudy
(2) hydrophytic, clear and cloudy

Fig. III-XXXII.

Daily Water Content, Transpiration, Temperature and Relative Humidity.

Fig. XXXIII.

Average Daily Water Content, Transpiration, Temperature, and Relative Humidity, Showing Seasonal Variation.

B = Water Content of *Fagus americana.*
W = Water Content of *Hamamelis virginiana.*
O = Water Content of *Quercus alba.*
b = Transpiration of *Fagus americana.*
w = Transpiration of *Hamamelis virginiana.*
o = Transpiration of *Quercus alba.*
t = Temperature.
r.h. = Relative humidity.

In Fig. III-XXXII transpiration values are multiplied by 10, and increased by 50.

In Fig. XXXIII transpiration values are multiplied by 10 and increased by 70.

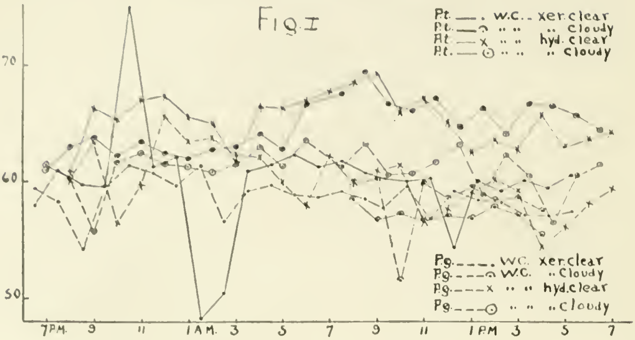
Fig. I

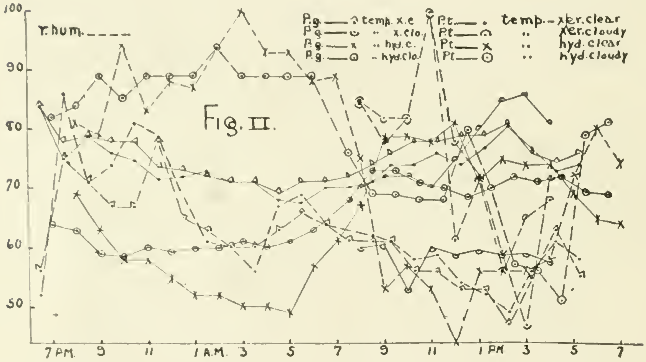
Fig. II.

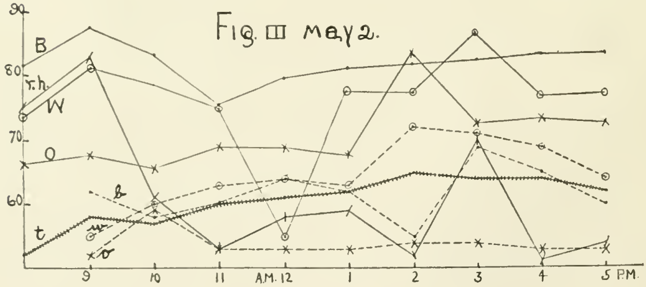
Fig. III May 2.

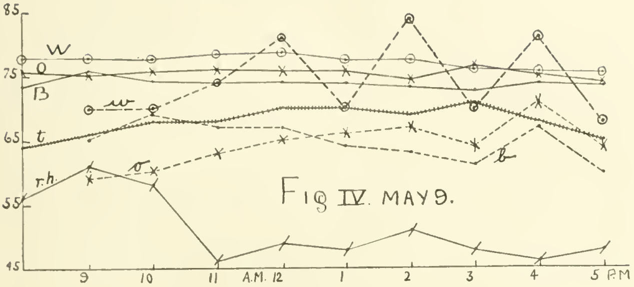
FIG. IV. MAY 9.

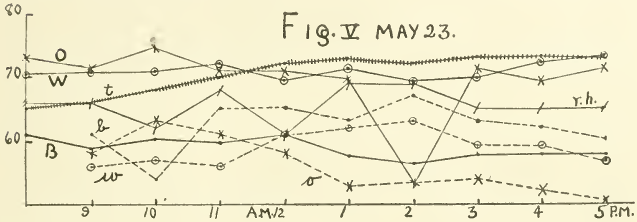
FIG. V MAY 23.

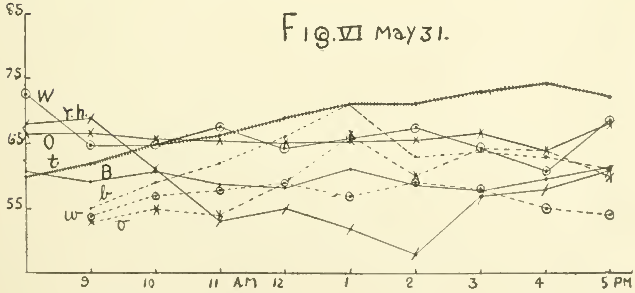
FIG. VI May 31.

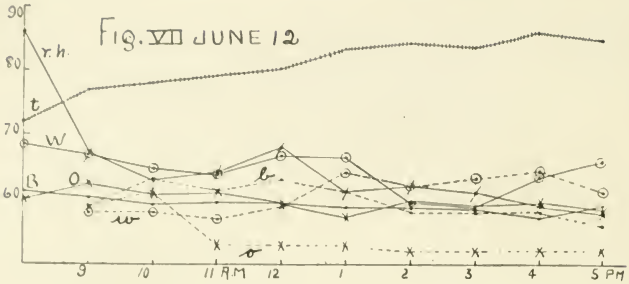
Fig. VII JUNE 12
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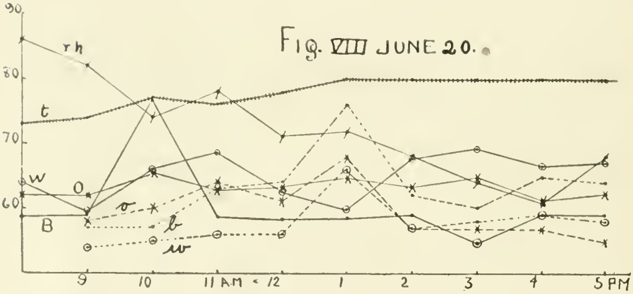
Fig. VIII JUNE 20.
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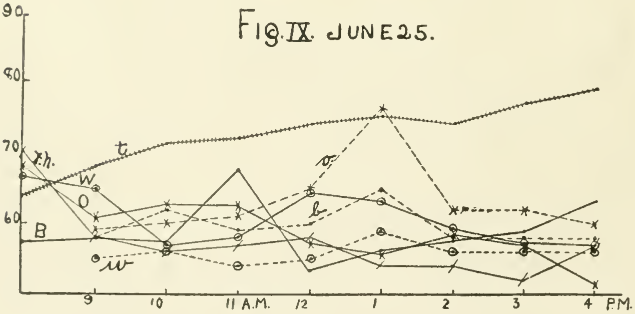
Fig. IX. JUNE 25.
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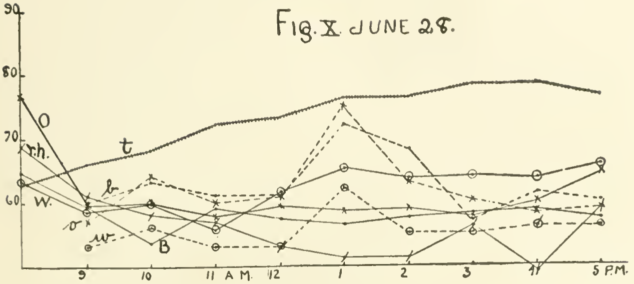

Fig. X. June 28.

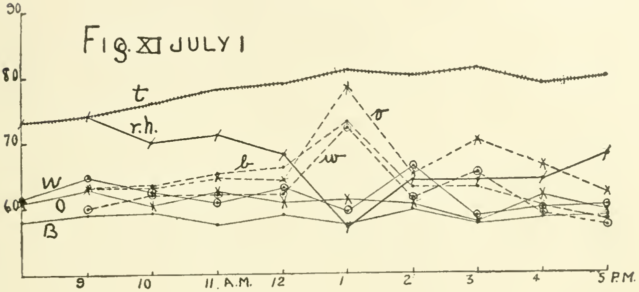

Fig. XI July 1

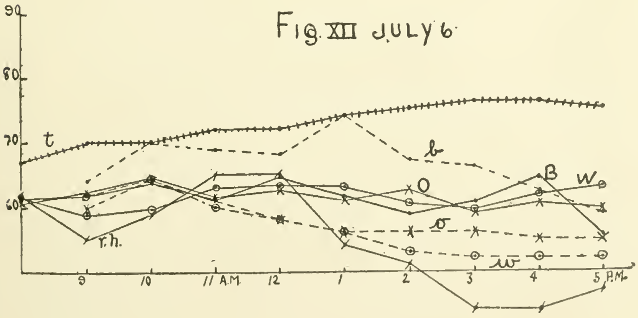

Fig. XII July 6.

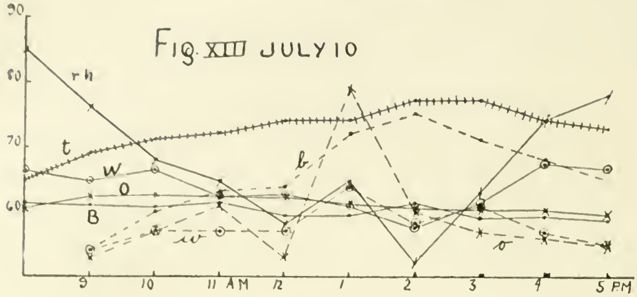
Fig. XIII JULY 10

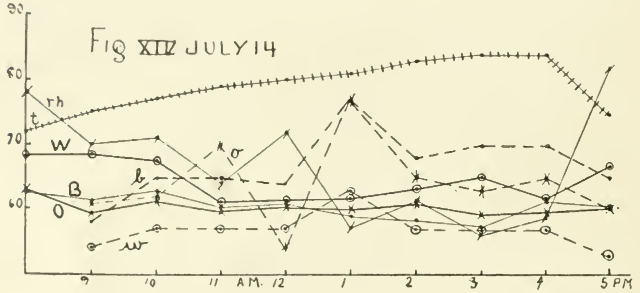
Fig. XIV JULY 14

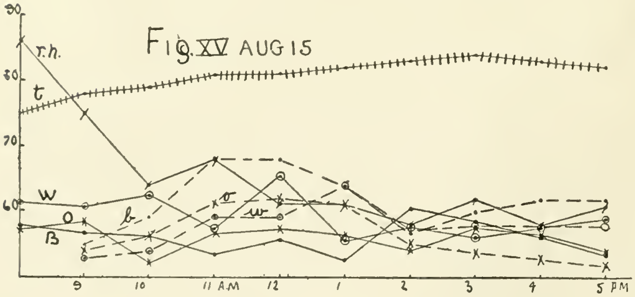
Fig. XV AUG 15

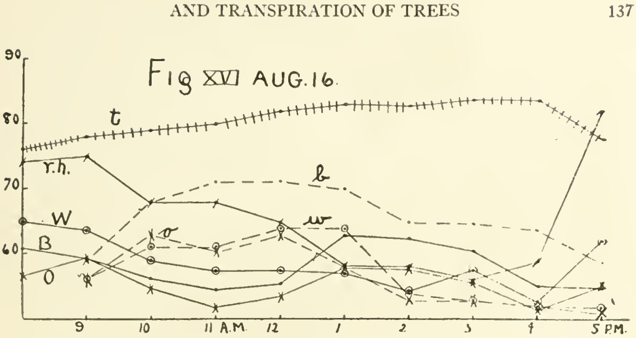
Fig XVI Aug. 16.

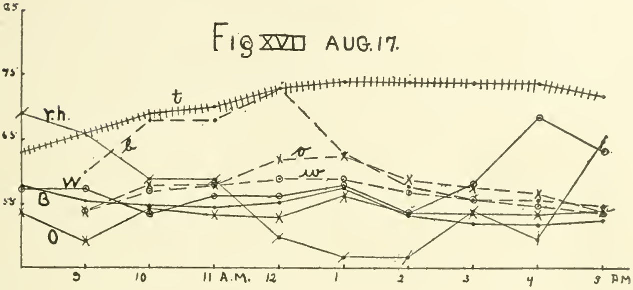
Fig XVII Aug. 17.

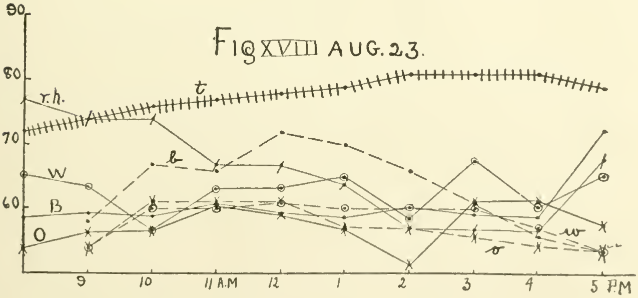
Fig XVIII Aug. 23.

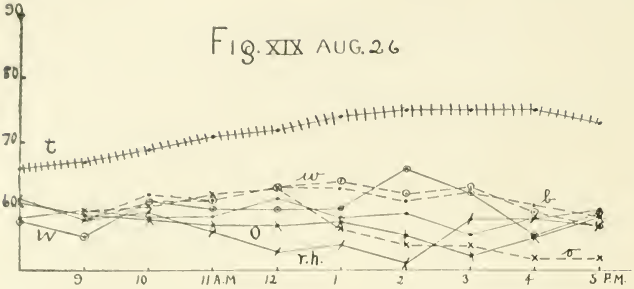

Fig. XIX Aug. 26

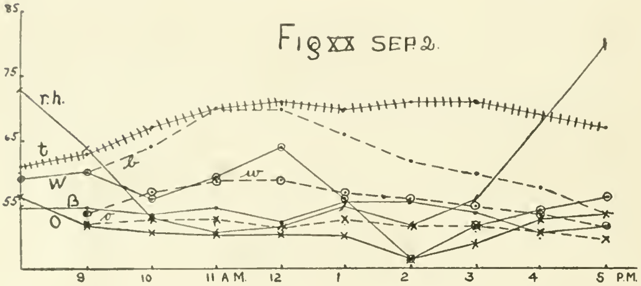

Fig. XX Sep. 2.

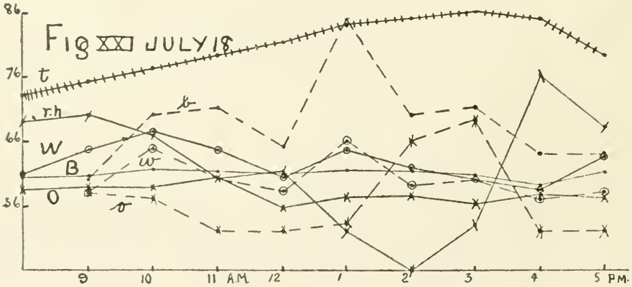

Fig. XXI July 18

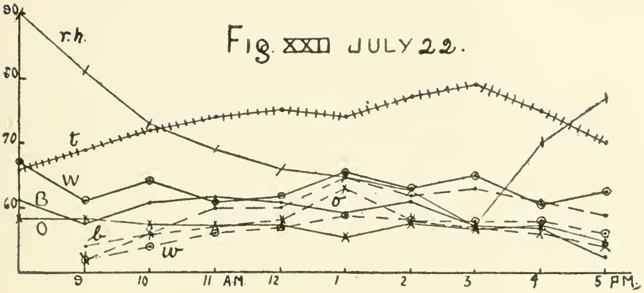

Fig. XXII JULY 22.

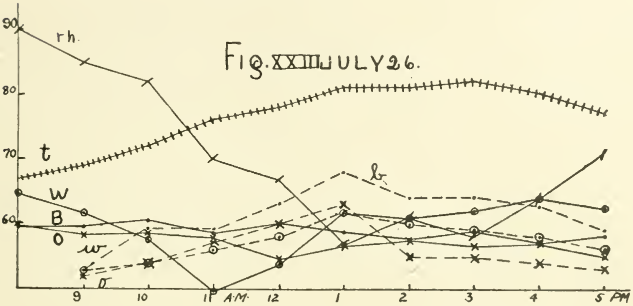

Fig. XXIII JULY 26.

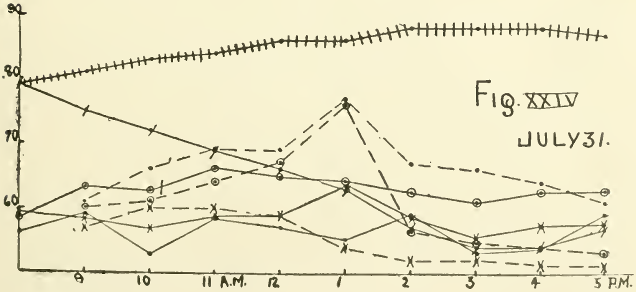

Fig. XXIV JULY 31.

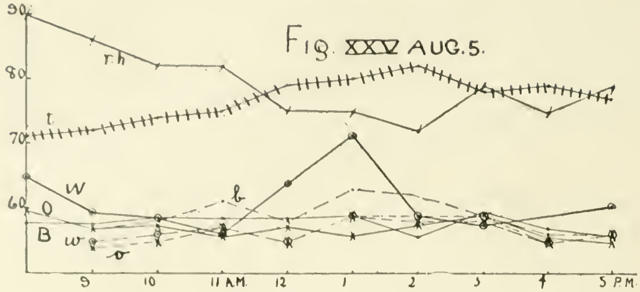

Fig. XXV AUG. 5.

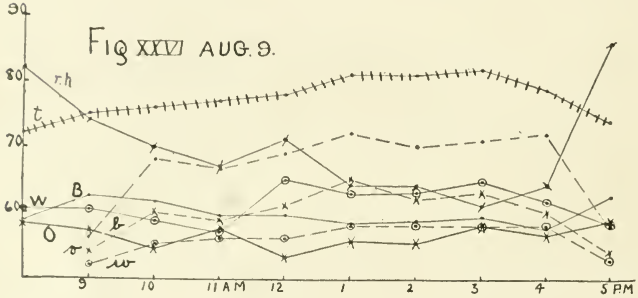

Fig XXVI AUG. 9.

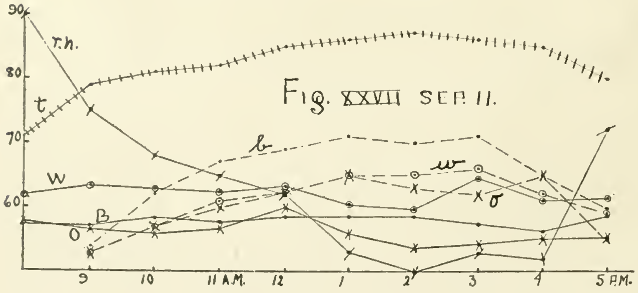

Fig. XXVII SEP. 11.

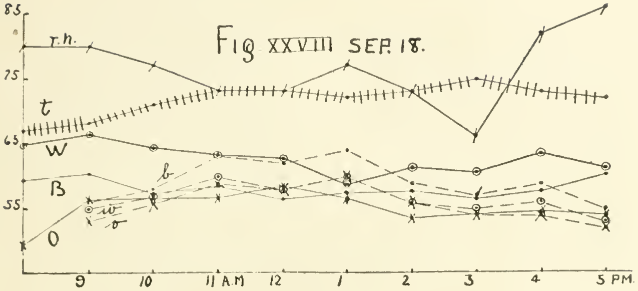

Fig. XXVIII SEP. 18.

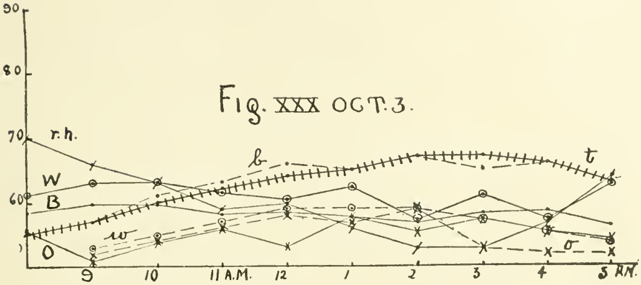

Fig. XXX OCT. 3.

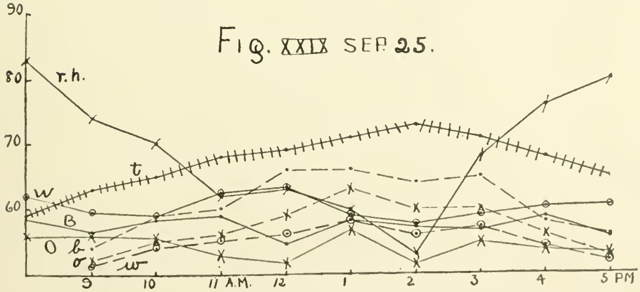

Fig. XXIX SEP. 25.

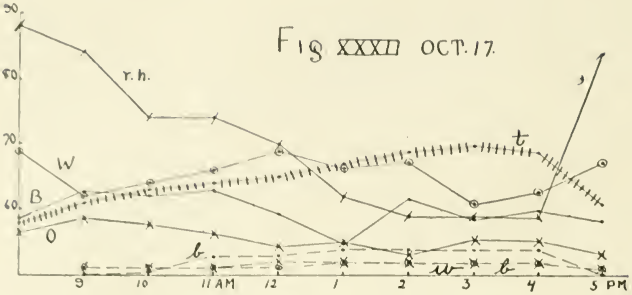

Fig. XXXII OCT. 17.

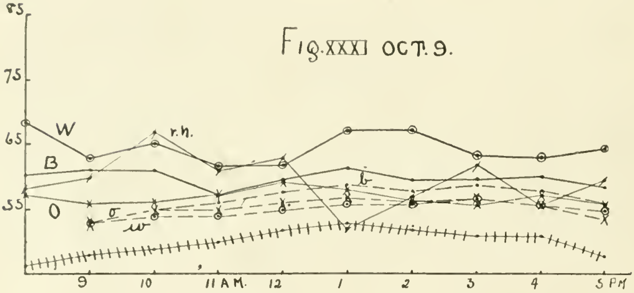

Fig. XXXI OCT. 9.

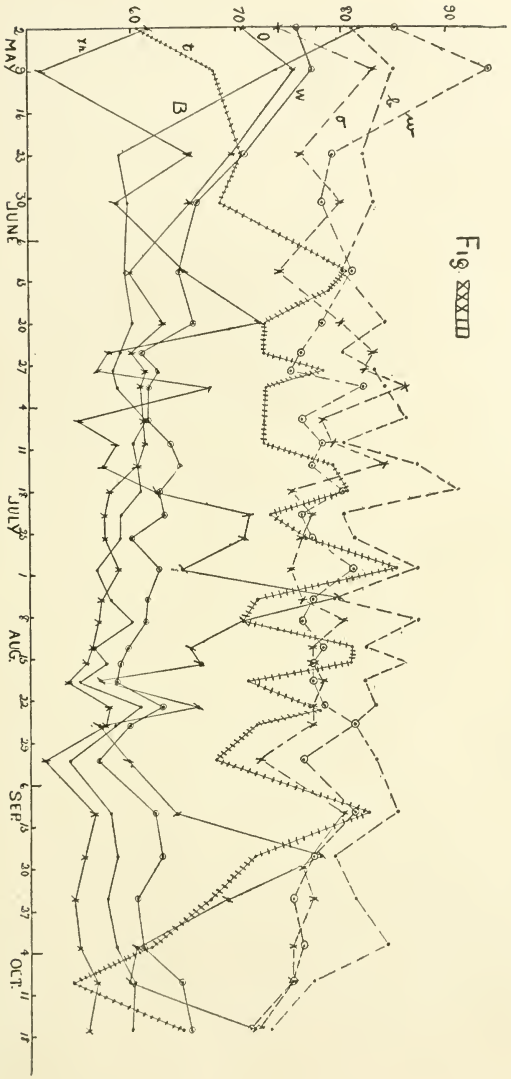
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Fig. XXXIII

THE DEVELOPMENT OF THE CHASMOGAMOUS AND THE CLEISTOGAMOUS FLOWERS OF IMPATIENS FULVA

BY

Franklin B. Carroll, M. A., Ph. D.
With Plates LV, LVI, LVII.

(Thesis presented to the Faculty of the Graduate School
in partial fulfilment of the requirements for
the Degree of Doctor of Philosophy).

I. General Description of Growth, Habits, Size of Plants, Habitat and Coloring

The Balsaminaceae include two genera, *Hydrocera* and *Impatiens*. *Hydrocera* is a monotypic genus of Asia. *Impatiens* includes 400 species mostly distributed through tropical Africa and Asia. A few species occur in the North Temperate Zone. Nine species of *Impatiens* occur in North America. *Impatiens fulva* is stated to occur in moist ground, from Nova Scotia to Oregon and Alaska, south to Florida and Missouri.

Impatiens fulva is usually described as a succulent herb, two to five feet in height. These figures represent an average height, but they do not include the whole range of variations generally met under different conditions. Under conditions which may be somewhat adverse, but not sufficiently adverse to cause any diminution in the productivity of the plants, lower plants are frequently to be found. In one locality that has been under observation for two years, the plants have never attained a growth of more than eight inches. The locality is a somewhat marshy piece of ground about twenty-five square yards in extent, at the foot of a hill, in close proximity to a spring and brook, and in rather heavy woodland shade. Even in dry weather the piece of ground remains rather marshy. In the two years that these plants have been under observation, they have produced only cleistogamous flowers, but these in abundance. Although rather pale green, these plants seemed perfectly healthy. The failure to attain greater height apparently is related to the bog conditions, perhaps the toxicity of bog water. To determine whether the height of these plants could be explained ecologically, several seedlings were transplanted in June 1916 to four-inch pots of drier soil. The average height of these transplanted specimens was one foot ten inches. The five plants which survived produced only cleistogamous flowers. The failure to produce chasmogamous flowers may be due to confined rootage, as will be noted below in discussion of further experiments. Several other similar localities were under observation during the summer of 1916. The average height attained by the plants was eighteen inches. They were characterized by the same pale green color, but seemed healthy and produced abundant cleistogamous flowers, but few chasmogamous flowers.

Short-stemmed plants are produced also in situations where the soil moisture is below the optimum for the species. At the top of the hill, above the plants just referred to, in an open woodland one such group of plants has been under observation for two years. During May and June the soil is quite moist, but in mid and late summer the soil becomes decidedly dry. During June and early July in both years, these plants were in good condition. They were of a darker green than the bog plants noted above, and some showed some evidence of the ruddy stems characteristic of the species. These produced abundant cleistogamous flowers during June, and moderately abundant chasmogamous flowers in July. They were killed by dry weather in August. The average height of these plants was two feet, and none were observed over two feet seven inches. Similar groups of plants in other sunny situations, where the soil was somewhat dry, were observed in fair abundance, averaging two feet or less in height.

Under the most favorable conditions relatively enormous heights are attained. The optimum conditions for vegetative growth seem to be found along brooks in rather open woodlands, with the roots of the plants washed by the running water. Under these conditions, plants six feet in height were found. The tallest plant measured in the vicinity of Philadelphia was six feet four inches in height. The stems and branches of these plants were a dense reddish color. The plants attained a circumference of over six inches near the base, and were buttressed by series of adventitious roots growing from the lower two or three nodes above the ground. Dr. Macfarlane reports plants of the species on Peak's Island, Maine, attaining a height of seven feet, and seven feet five inches. These plants were growing upon a sloping bank facing the sea. The abundance of percolating moisture and the open gravelly soil probably account for the enormous growth.

The habitat given by the Manuals for this species is moist shady places, in rich ground. The species is abundant, however, not only in this habitat but also in more sunny and drier locations. The optimum conditions seem to be a moderate amount of shade with moist, but well-drained ground. Plants growing in poorly drained or marshy situations behave as if under adverse conditions. The small plants already noted as growing under such conditions showed evidence of adverse enviroment in their height, color, foliage and flowers. The giant specimens noted above, on the other hand, cannot be said to grow under optimum conditions for the species, since the production of flowers was decidedly meager in most cases observed. The optimum conditions for flowering are not found when the running water is washing the roots of the plant unless they are situated in a slight shade, or even in the open sun. Much of the material used in investigating the histology was collected from several open sunny localities where the plants were never shaded, along several tributaries of Darby Creek, near Philadelphia. The plants were exceedingly crowded, three to four feet in height, and densely covered with flowers. In the other localities, the plants were close to running water on well-drained banks; all were characterized by exceedingly dense growth of plants and great abundance of flowers.

The species flourishes also in much drier situations. Plants were noted above as growing on a hill top in open woods; these were several hundred yards from running water or marshy ground. Along one of the drives in Fairmount Park, Philadelphia, a quarter of a mile from the nearest brook and in full sun, except in the later afternoon, a dense group of plants was kept under observation during the summer of 1916. The plants were thirty to thirty-six inches in height and produced both cleistogamous and chasmogamous flowers in abundance. On a sunny bank in the botanical gardens of the University of Pennsylvania numerous plants have maintained themselves for several years. These are stocky plants averaging four feet eight inches in height and five inches in circumference. From the lower nodes there are circles of adventitious roots. These plants bear abundant, though rather small, chasmogamous flowers during August. All these plants noted grow in rich soil, but the species is also to be found in poorer, sandy soils. It is met with in the sands of the New Jersey Pine Barrens. Experimentally also the plants were grown in poor soils. Seedlings transplanted to five-inch pots of sand maintained themselves quite as well as those transplanted to pots of rich soil, although they produced as a rule fewer chasmogamous flowers.

The color of flowers and stems is subject to considerable variation. The flowers are typically yellow to orange-yellow dotted with brownish red. The yellow color is born by plastids, while the red is due to a dissolved pigment in the cell sap. It is chiefly on the petals and on the inside of the large spurred sepal, to a lesser extent on the anterior haired sepal that the brownish-red coloring occurs.

The seventh edition of Gray's Manual mentions the occurrence of spotless forms. Such forms seem to be rare. Not uncommon are forms with only a few, five or six, small reddish spots inside the spurred sepal and an equal number on the petals, the anterior sepal bearing only one or two spots. From this condition, gradations may be traced to forms with densely spotted anterior and posterior sepals, and petals with the spots confluent in brownish-red masses. Plants with white flowers spotted with brownish black are occasionally met with. It is stated in Britton and Brown's Manual that flowers nearly white unmottled occur. The coloring of the flowers seems to be definite for a strain of plants, plants raised from seed develop the coloring of the parent. The ruddy coloring of the stems, however, does not seem to be so definite a genetic character, but dependent to an extent upon ecologic conditions. Plants grown under somewhat adverse conditions are apt to show greenish stems without the red markings. Under optimum conditions for vegetative growth, as in partial shade with abundance of running water, the stems become dark red.

Dates of Appearance and Duration of cleistogamous and chasmogamous Flowers

The dates of appearance and duration of chasmogamous and cleistogamous flowers vary considerably with the locality and conditions. In the vicinity of Philadelphia, the earliest date noted for the appearance of chasmogamous flowers in 1916 was June 20th. They appeared in abundance by June 24th on a group of plants growing in the open sun in a dry roadside situation. The dryness of the soil and consequent higher temperature may explain their early appearance. The period of maximum production began a week or ten days later. Plants in moister situations were relatively late in blooming. One group of plants under observation situated in a rather densely shaded situation at the edge of a marsh did not produce chasmogamous flowers until early August. The period of blooming extended to the last days of September and in scattered instances to early October.

The cleistogamous flowers appear early in June in the vicinity of Philadelphia and last through the growing season. This statement does not agree with several published accounts. Miss Riatt[39] states for

Impatiens pallida that the cleistogamous flowers appear in May and last through June, a few lasting throughout the summer. Bennett[4] states for *Impatiens fulva* that on the banks of a tributary of the Wey the two kinds of flowers were "absolutely synchronous." Bennett quotes Weddell as reporting for *I. noli-tangere* that the inconspicuous flowers occur latest, and Mohl as reporting only cleistogamous in June, and only chasmogamous flowers in September. He reports plants of *I. noli-tangere* observed in the Botanic Gardens at Oxford on the last day of September as having "abundance of cleistogenous flowers with half-expelled corolla-cap, while scarcely any of the perfect flowers were met with and these on different plants." The earliest date noted in the vicinity of Philadelphia in 1916 for the appearance of cleistogamous flowers in *I. fulva* was June 10 and in *I. pallida* June 13th. Bennett says "I have never found the two kinds on the same branch, occasionally on different branches of the same plant, but more often on separate plants." In southeastern Pennsylvania in both species the cleistogamous flowers were found in abundance along with the chasmogamous flowers through the entire summer until late September. In Potter County, Pa., and in Algonquin Park, Ontario, both types of flowers were found in abundance during August. Indeed one of the most noticeable facts about the cleistogamous flowers is their universality. They are less abundant in mid-summer on the taller and more vigorous plants, but on the extremely tall plants noted above chasmogamous flowers were also generally less abundant. Smaller plants bear the cleistogamous flowers more abundantly, yet plants of average height growing under good conditions were found to bear both kinds in fair abundance, the cleistogamous flowers appearing upon the shorter and lower branches. Even in mid-summer careful examination of lower, short side-branches of plants in full chasmogamous flower showed the presence of the cleistogamous flowers and fruit.

Pseudocleistogamous Flowers

In addition to the truly cleistogamous flower, an intermediate type, or rather various intermediate grades between cleistogamous and chasmogamous, are to be found. Bennett says that he never noticed the least indication of any intermediate condition between the two kinds of flower (4 p. 152). He states that Bentham and Boswell-Syme describe the two kinds of flowers as growing intermixed in the same raceme in *I. noli-tangere*. I have found intermediate types in both *pallida* and *fulva* in the same inflorescences with normal chasmogamous flowers.

These flowers are considerably larger than the typical cleistogamous flowers. The cleistogamous flowers at the stage of fertilization average two millimeters. Their morphology is that of the chasmogamous flowers. The four sepals are present in the same position and form. The anterior sepals show the same double appearance; the posterior has the well-developed spur; the two lobed petals are fully developed, the whorl of stamens appears fused and each stamen bears four pollen sacs like the chasmogamous, but unlike the cleistogamous flowers which have only two pollen sacs. They could not be distinguished from chasmogamous buds of the same age. Apparently these flowers are developing to be chasmogamous flowers, but fertilization occurs before the bud opens. The whole mass of calyx, corolla and stamens is then loosened, and as the pistil expands this mass is forced off. Some of these flowers were marked with tags at the stage when loosening of this mass was first evident. Subsequent observations showed that the majority of such marked flowers developed fruit and seed similar to those of chasmogamous flowers.

These intermediate flowers belong to the type which Hansgirg (20) has designated pseudo-cleistogamous. Morphologically the flowers are like the chasmogamous, but physiologically they behave as cleistogamous flowers. Adverse conditions of light, temperature, moisture, or drought apparently lead to self-fertilization in buds. Hansgirg distinguishes photo-, hydro- and thermo-cleistogamous flowers. These do not include all the causes which lead to this phenomenon. Numerous references occur through the literature to the occurrence of the phenomenon under varying conditions and in numerous species. Partial lists of such species and conditions are given by Hansgirg (20) and Knuth (26 I pp. 55-58 English Translation). In the case of *I. fulva* and *pallida* observed in late August and early September 1916, they were probably induced by dry weather in August. None was observed during July of that year before the dry spell began, but they were not rare toward the close of the dry weather and for a short time succeeding. In none of the experiments described below, however, in which plants were subjected to various adverse conditions, were pseudocleistogamous flowers detected.

Still another type of flower which would fall within Hansgirg's group of pseudocleistogamous flowers, was discovered in castration experiments described below. Full grown buds just about to expand were cut open. The pollen sacs were found already ruptured. The stamens were removed, and the flower covered to prevent the entrance of insects.

In several of the rather small number of experiments, these flowers produced good seed. This would indicate that self-pollination had occurred before the removal of the stamens. How far this self-pollination before the opening of the bud occurs in nature would be difficult to determine. There is no indication of the occurrence in the behavior of the flower. The castrated flowers were normal chasmogamous flowers to all appearances. Apparently they would have opened their sepals and petals within another day. In the light of the possibility of self-pollination at this stage and the subsequent opening of the sepals and petals, one could not say with certainty whether a particular flower was pollinated before or after it opened.

Pollination and Fertility of cleistogamous and chasmogamous Flowers

References occur in the literature to a lack of agents of pollination and to a high degree of sterility among the chasmogamous flowers of *I fulva.* Bennett (4) says "the conspicuous flowers are stated by all observers to be usually barren." This does not agree with my own observations. It is quite true as Gray states (18) that "many of the larger and fully developed flowers fall away without forming fruit." In one large group of plants under observation, not one chasmogamous flower was observed to produce fruit. Yet the abundant production of fruit from chasmogamous flowers was elsewhere evident. A large number of flowers were marked with tags and observations carried through till the collection of seed. Bennett thinks the arrangement of stamens and pistils is such as to prevent self-pollination, yet equally adapted to prevent cross-pollination. The filaments send out from their inner faces broad processes which are connivent over the pistil in such manner as to prevent any pollen from the anther reaching the pistil. The whole whorl of anthers later falls away together, exposing the stigma. Bennett states that he has never observed this to take place spontaneously, but that the flower drops as a whole carrying the pistil along. This phenomenon frequently occurs, but it seems to be an abnormal occurrence. Normally the whorl of stamens falls away exposing the pistil. The petals and sepals fall several hours to a day later. The falling away of the flowers with the pistil included, observed by Bennett on the Wey may be associated with conditions of a foreign home. Under adverse conditions in America the same process occurs. One group of plants, nearly denuded of foliage by insects, showed this phenomenon and similar instances were observed in dry weather.

The agents of pollination in the vicinity of Philadelphia seem to be chiefly bumble-bees and humming-birds. Bennett says he never saw the flowers visited by insects. He quotes a curious old work, "New England's Rarities discovered in Birds, Fishes, Serpents, and Plants of that Country," by John Josselyn, Gent., London, 1675, in which occur a drawing and a description of apparently *Impatiens fulva* under the title of the Humming-Bird Tree. Dr. Macfarlane reports stands of *Impatiens fulva* on Peak's Island and also on Mount Desert, Maine, that were regularly worked over by a group of humming-birds. Bees were not often seen to visit these flowers. In the Philadelphia vicinity, humming-birds may frequently be seen visiting the flowers, but the most persistent visitors seem to be bumble-bees. Smaller bees and flies may sometimes be seen, but the bumble-bees are found visiting flower after flower in continuous succession. The bee approaches the flower as it hangs upside down, alights upon the broad lamina of the petals and makes its way back to the inside of the spur, brushing its back against the mass of stamens above. Bees emerging may be seen to have their backs covered with pollen. The same day the flower opens, or on the succeeding day, the mass of stamens falls away leaving the pistil exposed in the flower. These detached whorls of stamens are to be found lying about. A bee subsequently making its way into the flower must brush its back against the expanded stigmatic surfaces. Müller (36) gives a similar account of pollination. Not infrequently bees may be seen alighting upon the outside of the spur and making their way to a point on the outside near the nectary, then on leaving to visit another flower in the same manner. I have occasionally found small flies working over the flowers in the same way, the flowers visited sometimes showing perforations and sometimes none in the spur. Only a minority of the bees visited the flowers in the above manner, but the bees following this method were not seen to enter the spur through the open end. It was not determined whether the bees following this method were a species different from those entering through the mouth of the spur. Trelease (48) reports observation of this occurrence. He determined that the bees did not make this perforation but merely took advantage of perforations already existing.

The method of pollination described for *fulva* applies also for *pallida* and *sultani*. Somewhat different methods are reported for other species. In *I. noli-tangere*, according to Knuth, (26) the pistil protrudes between the staminal processes and so may be pollinated. In *I. parviflora* and *I balsamina* the same mechanism is reported (Knuth 26). In *I.*

glanduligera, Landl. (= I. Roylei Walp) Loew (30a) has described a different mechanism. A cleft between the anterior stamens forms a passage through which the hairs on the back of the bumble-bee are thrust as the bee makes its way below the stamens. The staminal processes aid in the function of collecting the pollen forming a "pseudo-stigma." According to Knuth, (26) Hildebrand and Delpino (cited by Knuth), however, the mechanism is in this species much as in the preceding species described.

Accounts differ as to the fertility of the various species of *Impatiens* with their own pollen. In the Gardener's Chronicle for 1868 a quotation from "American Gardening" states that the "Balsams, (*Impatiens pallida* and *fulva*) cannot be fertilized with their own pollen." This does not agree with Darwin's account or with my own experiments. In a personal communication from Darwin, quoted by Bennett, (4, 152), Darwin gives the following account—"Eleven such pods from perfect flowers, spontaneously self-fertilized yielded on an average 3.45 seeds. I carefully brushed away the pollen from some of the perfect flowers, and fertilized them with pollen from a distant plant, but got only three pods, containing to my surprise only 2, 2 and 1 seed." Darwin (12, p. 366) states that *Impatiens barbigera* set seed abundantly when covered with a net, and that plants of *I. noli-tangere* covered with a net produced from perfect flowers eleven spontaneously self-fertilized capsules which contained on an average 3.45 seeds (12, p. 367).

My own experiments in self-pollination included *I. fulva*, *pallida* and *sultani*. Each proved fertile to its own pollen. Each was tested by two methods, the first to determine the occurrence of spontaneous self-pollination, the second to determine the results of the direct application of pollen. In the first method the flowers were sealed in paper envelopes to exclude insect visitors. In five experiments with *sultani* no seed was set. In the greenhouse where were some two dozen pots of *I. sultani* no seed occurred during the winter except as a result of hand pollination. When cross-pollinated by hand they set abundant seed. In five flowers of *fulva* sealed to allow spontaneous selfing, three set seed, and in five flowers of *pallida*, one set seed. In the second method to test the effects of direct application of the pollen, a loosened whorl of stamens was removed, or one was taken that had fallen from position but lay on the petals of the flower, care being observed to exclude possibility of cross pollination, and the pollen was applied to the stigma. Five experiments on each of the species resulted as follows: in *pallida* four set seed; in *fulva* five; in *sultani*, five. In *pallida* and *fulva* some flowers were castrated just before the opening and were sealed in paper envelopes. In some of these the pollen sacs had already ruptured. In *pallida* the results of five experiments were as follows: one capsule with five seeds; one with four; one with three; and two failed to form fruit. Somewhat similar results were obtained with *fulva*; unfortunately the records of these have been lost. The results with these castrated flowers would indicate that spontaneous self-pollination sometimes occurs before the opening of the flowers.

The number and size of the seeds in cleistogamous and chasmogamous capsules, Miss Riatt (39)states to be the same in *I. pallida*. So far as the number of seeds in the capsule is concerned, this does not agree with my observations on either *pallida* or *fulva* In *pallida*, I have found in the vicinity of Philadelphia the number of seeds in the chasmogamous capsules is generally four or five; in the cleistogamous capsules two or three, or not uncommonly on the lower branches only one. This number represents also the condition in *fulva*. My observation of the sizes of the two types of seeds in *fulva* agrees with Miss Riatt's report of *pallida*. The average size in the chasmogamous seed was 5.1 × 4 m. m. and in the cleistogamous seed 5 × 4 m.m. The weights varied with the condition of drying. In seeds gathered from the bursting pod the average weight in 48 chasmogamous seeds was 0.0208 grams and in 59 cleistogamous seeds was 0.0195 grams. The size of capsules is quite different in the two types of flowers, but the smaller capsule of the cleistogamous flowers is accounted for by the smaller number of seeds.

There seems to be no general rule in the different species as to the size and number of cleistogamous as compared with chasmogamous seed. Shaw (45) says for *Polygala polygama* that there is no difference in the seed resulting from the different types of flowers. Helene Ritzerow (41, p. 71) quotes Burck (11) to the effect that in *Heteranthera spicata* the cleistogamous fruit is a half larger than the chasmogamous and in *Heteranthera Potamogeton*, Solms, and *Heteranthera Kotschyana*, Fenzl, it is more than twice as large with twice the number of seeds; in *Commelina bengalensis* (41 p. 172) the seeds of the cleistogamous flowers are double the weight of those of the chasmogamous flower. In *Vandellia nummularifolia* (41, p. 201) the number of seeds in the cleistogamous flowers is on the whole lower than in the chasmogamous. In *Houstonia caerulea* (41, p. 204) the size of the seed is larger in the chasmogamous capsule, but the number of seeds per capsule is larger in the cleistogamous capsules. In *Specularia perfoliata* (41, p. 208) the number of seeds was greater in the chasmogamous form. The relative number and size of the two types of seed are thus seen to vary considerably among different species.

II. Experiments and Observations on the Production of chasmogamous and cleistogamous Flowers

Observations in the field on the production of cleistogamous flowers show a wide range of conditions under which they appear. In general as was noted above, conditions which favor greatest vegetative growth, lead to small production of both kinds of flowers. These conditions seem to be rather dense shade and rootage in soil saturated with fresh running water. Along brooks in light shade or open sunny meadows the plants are shorter and produce chasmogamous flowers in greatest profusion. Before the production of chasmogamous flowers these plants are quite generally to be found bearing abundant cleistogamous flowers and careful examination of the plants during the period of maximum production of chasmogamous flowers will frequently show shorter shaded branches on the lower parts, and these bear cleistogamous flowers. In one such situation where every one of twenty-five plants was examined the cleistogamous flowers were found in the act of pushing off the perianth cap or these caps were found lying on leaves or other parts of the plant. In one station by an open sunny roadside that was under observation during the last week of July and the first week of August, and again during September 1916, chasmogamous flowers and seed were produced in abundance, and cleistogamous flowers which were abundant during June and early July continued more sparingly in the later weeks. Under adverse conditions the production of chasmogamous flowers is reduced, but that of cleistogamous flowers remains constant as in the early part of the season, or increases. In a station on a hillside in the open woods that was under observation during the spring and summer of 1916, the plants produced abundant cleistogamous flowers from the middle of June to the middle of September. During July and August a few chasmogamous flowers only were produced. The soil during June was rather moist, but during the later summer was quite dry for a depth of two inches. In a bog in the woods at the foot of this hill, pale green plants eight to ten inches high lasted through the summer, producing cleistogamous flowers very sparingly and chasmogamous flowers not at all. Conditions here were very similar to those in the bog noted above. Four such boggy situations in the woods were under observation during the entire summer. The conditions were similar in all—a small, stagnant, poorly-drained spot in rather dense shade. The plants in each were less than a foot in height, of a pale green color, and producing rather sparingly cleistogamous, and no chasmogamous flowers. The adverse condition here would seem to be the toxicity of the bog water. Plants in the same condition of shade, but in well-drained soil attained heights of two and a half to three and a half feet, with darker green, or somewhat reddish stems, and chasmogamous flowers. Conclusions derived from observation of field conditions are that under the optimum condition for vegetative growth both kinds of flowers are rare; under conditions optimum for the production of chasmogamous flowers, cleistogamous flowers are produced in abundance in the early moister part of the season and more sparingly throughout the season; under adverse conditions of drought or toxicity of bog soil only, or chiefly, cleistogamous are produced.

Goebel (16) performed a series of experiments upon several plants including *Impatiens noli-tangere* to determine the conditions of production of cleistogamous flowers. He planted *Impatiens noli-tangere* seeds in pots of earth and sand. Some of the sand pots he watered with nutrient solutions. The plants in earth produced both chasmogamous and cleistogamous flowers; those in sand, only cleistogamous. Those watered with nutrient solution produced more abundant cleistogamous flowers, but no chasmogamous. He reported that plants of this species, attacked by *Sphaerotheca Castagnei* and suffering loss of leaves in July while in chasmogamous flower showed on August 11th only cleistogamous flowers, while healthy plants of the species were still in chasmogamous flower. He cites also lighting and high temperature as leading to the production of cleistogamous flowers.

My own experiments on cleistogamous flowers aimed to determine the production of the two kinds of flower under adverse conditions of drought, nutrition, leafage and temperature. Young plants averaging 3.5 dm. in height were transplanted from the woods to pots on May 29th. Plants were marked in the woods to serve as controls. To test the effect of drought eight pots were selected, four in rich soil and four in white sand. These were watered sufficiently to keep the plants on the point of withering. One plant in sand died within a week without producing flowers; one died within two weeks after producing a few cleistogamous flowers; two continued to live for a month producing cleistogamous flowers sparingly. Of the plants in the soil one died in a few days without flowers; two died before the end of the third week after producing a few cleistogamous flowers; one lived over a month with a sparse production of cleistogamous flowers. No chasmogamous flowers were produced. The plants in the soil were somewhat healthier probably because of the greater retention of moisture by the earth. To determine the effect of high temperature, two pots were kept in a greenhouse with temperature 15° F. higher than outside. One plant succumbed very quickly, the other lived twenty-two days in the house and produced three cleistogamous flowers. To determine the effect of shade, two pots were placed in dense shade, where they continued in good health for six weeks producing cleistogamous flowers continuously. To test the effect of soil nutrition, two pots in white sand were watered with Pfeffer's nutrient solution, two in white sand received only pure water and two in soil served as controls. One of the pots in soil produced chasmogamous flowers; all the others produced only cleistogamous flowers. To determine the effect of reduced leafage, plants in the woods from which the potted plants had been taken, were stripped of all except two or three leaves. Two died early, three continued to produce occasional cleistogamous but no chasmogamous flowers. The controls in the woods produced abundant cleistogamous flowers during June and July. During July, they produced chasmogamous flowers rather sparingly. A dry spell in August killed practically all of the plants.

The results of experiments are in accord with the facts ascertained by field observation. Cleistogamous flowers are generally produced in the early moister part of the season on the majority of plants, even those living under optimum conditions. They may continue under even optimum conditions through the season along with chasmogamous flowers. Adverse conditions of drought or nutrition, directly through failure of necessary elements, or indirectly through lighting, or through reduction of leafage by mechanical agents, or the attack of parasites, or toxic conditions of soil, may lead to the suppression of the chasmogamous flowers. The cleistogamous flowers will then continue, or will increase in numbers.

III. GENERAL ORGANOGENY OF THE CHASMOGAMOUS FLOWER

The development of the flower of *Impatiens pallida* has been described by Miss Riatt (39). Her account differs somewhat from the conditions in *fulva*. In the early stages the conditions are the same in both cleistogamous and chasmogamous flowers. Miss Riatt states that the development of the flower begins with the appearance of the saccate sepal. This is not the case with *Impatiens fulva*. The first member to arise is the bract (Fig. 1 A, Fig. 7 A). A little later the two lateral sepals appear (Fig. 2). Next the posterior saccate and then the anterior sepal (Fig. 3). Figure 7 is a longitudinal section of a cleistogamous flower, but the relation of parts here shown is exactly the same as in the chasmogamous flower. Miss Riatt's Fig. 1 A is a longitudinal section of a stage slightly older than the older stage of Fig. 7. The member she marks saccate sepal looks very much like the section of the bract. The lateral sepals would not appear in this section. The transverse sections (Figs. 1, 2 and 3) show the relation of the bract and sepals. Payer's account of *Impatiens Royleana* agrees with the above account of *fulva*. The petals appear as two prominences inside the sepals (Fig. 4 E). These are the posterior lobes. A little later the anterior lobes arise (Fig. 6). For a time these prominences arise separately; later they join in pairs, and each prominence then rises to form the single claw of a two-lobed petal. The stamens appear about the same time (Fig. 4 F and Fig. 8 C). At first the stamens are entirely free, and at a later stage they become joined in their anther wall and filaments. Three papillae inside of the stamens mark the developing pistil (Fig. 5 A). The pistil is still quite open when the ovules are well advanced, and it never entirely closes, stylar canals remaining open to receive the pollen tubes. The stylar canals are formed here not by breaking down of tissue, but through failure of the pistil to entirely close over or fuse. Figure 33 shows pollen tubes passing through the stylar canals in a cleistogamous flower. This figure, except for the anthers and calyx above, would serve equally well for the chasmogamous flower. The placenta arises from the floor of the carpellary cavity, and the septa push inward from the carpellary walls to meet the placenta (Fig. 6). The placenta would thus seem to be axial in origin, and the ovules arising upon it, cauline ovules.

Morphology of Petals and Sepals in the chasmogamous Flower

At one time there was considerable controversy as to the morphology of the petals and sepals in the genus *Impatiens*. Payer (37) summed up the various views up to his time. Jussieu had attributed to the flower of *Impatiens balsamina* two small lateral sepals, and four petals of which the inferior was spurred, the superior hooded and the two lateral deeply bilobed. Richard viewed the flower as having four sepals—two small lateral, one spurred inferior, and one superior—and four petals which had become joined in pairs. Knuth viewed the calyx as composed of the two small lateral sepals, of a spurred inferior sepal and a superior formed of two joined, and the corolla as composed of four petals joined in pairs, the fifth being aborted. Bernhardi viewed the two small green sepals as two bracts. The calyx to him was composed of five members—an inferior spurred sepal, a superior sepal composed of two joined, and two little sepals which usually aborted and which botanists had not mentioned. The corolla was composed of five petals—four joined in pairs and a fifth which had united with the two joined superior sepals. Roeper viewed the calyx as composed of five parts—two were often rudimentary or disappeared, two were small lateral sepals, and the fifth spurred sepal was toward the axis of the inflorescence. The corolla was composed of five petals which alternated with the sepals—four were joined in pairs, the fifth was the superior petal of the older botanists. Payer accepted the view of Roeper, and agreed that the two small prominences representing the anterior sepals were to be found in the young stage of *Impatiens balsamina* and figured them in the young stages of *Impatiens Royleana*. Lindley (28) followed the view of Knuth. Henfrey (22) arguing from the morphology of double balsams adopted Roeper's view although he failed to find the rudiments of the two anterior sepals reported by Roeper and Payer in *Impatiens balsamina*. The view of Roeper was adopted by Warburg and Reiche in "Pflanzenfamilien." Gray's (18) view agreed with that of Knuth, and this was reported in the seventh edition of Gray's Manual. Britton and Brown's Manual followed the view of Roeper. The double appearance of the anterior member in our native species would seem to suggest that it is formed of two members which if it be regarded as belonging to the petaline whorl would raise the number to six members, but if it be regarded as belonging to the sepaline whorl would raise the number to five. The occurrence occasionally of two spurs upon this structure would further emphasize its double nature. The fact that supernumerary spurs occur upon the two lateral usually greenish sepals, but not upon the two-lobed petals would tend to argue its connection with the sepaline whorl. The occurrence in other species of the genus of spurs on all five structures further argues for this view. In this paper, I have therefore adopted provisionally the view of Knuth and Gray in describing the calyx as composed of four sepals, the anterior sepal being formed of two fused sepals and the corolla as composed of two-lobed petals representing four petals fused in pairs. These have been well figured by Gray (18).

Peloria is not uncommon in the genus *Impatiens*. It is stated that the two lateral sepals are often spurred in double garden balsams.

Masters (32) states that the balsams become peloric by the addition of spurs. Penzig (38) takes the view that three sepals are present. The two missing sepals sometimes reappear as small leaflets. In *I. glandulifera,* all five sepals are present. Sometimes the side sepals are spurred and sometimes all five when present. Britton and Brown (18) state in *Impatiens fulva* that "spurs are occasionally developed on the two small exterior sepals, and spurless flowers have been observed." I have found in *Impatiens fulva* not rarely spurs on the two small lateral sepals. These spurs are generally much smaller than the posterior spur. Occasionally the anterior sepal is found with the two spurs developed. These conditions seem to be common to all flowers on the plants showing them. These supernumerary spurs may develop in such manner as to prevent the entrance of insects. The plants must then depend upon the cleistogamous flowers, or upon self-pollination in the chasmogamous flowers.

General Organogeny of the cleistogamous Flowers; Morphology of Petals and Sepals

The general organogeny of the cleistogamous flowers is the same as that of the chasmogamous flowers up to the stage of the differentiation of the spur in the posterior sepal. Figures 7, 8, 9 and 33 represent the conditions in the cleistogamous flowers. Bennett figures and describes 4, p. 148) a difference between the buds of the two kinds of flower. "The bud of the conspicuous flower (fig. 1) has the apex of the two exterior (lateral) sepals hooked, while in that of the inconspicuous flower (fig. 2) it is straight, the two buds at this stage being nearly equal in size. The removal of the two exterior sepals shows a still greater difference (figs. 3 & 4), the spurred posterior sepal or nectary being very easily seen in the former case (fig. 3), while in the latter (fig. 4) the interior whorls of organs are, as described by Prof. Gray, "nearly regular but never developing beyond a very minute size." In earlier stages, before the differentiation of the spur, this difference could not be detected; cross sections, however, through the stamens, and especially through the pollen sacs will prove the type of flower.

In the mature cleistogamous flower all parts are present that are present in the chasmogamous flower. Miss Riatt (39) says that the petals are aborted in the cleistogamous flowers of *Impatiens pallida*, and Britton and Brown (8, p. 304) state for the family that the cleistogamous flowers are apetalous. Knuth reports them present in the cleistogamous flowers of *I. noli-tangere* (8, p. 52). They are evident in *I. fulva* (Fig. 9). Gray (18) figures them in his dissection of the cleistogamous flowers of *fulva*. They show the same two lobed structure as the chasmogamous petals. The four sepals are present in the same position as in the chasmogamous flowers (Fig. 9). The spur sometimes appears as a prominence, but sometimes no trace of it could be detected. Darwin, quoted by Bennett,[4] speaks of the "nectary in the cleistogamous flowers as a mere rudiment." Five stamens are present but differ from the chasmogamous stamens in a manner to be explained below. The ovary is five-celled at first, but becomes one-celled at maturity through the breaking down of the septa.

The condition in *Impatiens fulva* must not be regarded as typical for cleistogamous flowers in general. In many there is reduction in either calyx or corolla, or in both. Helene Ritzerow[41] lists a number of species suffering such reduction. She states that in *Specularia perfoliata* the number of sepals is reduced; in *Cardamine chenopodifolia* and *Helianthemum glomeratum* the corolla is wanting; in *Viola sp.*, *Polygala sp.*, and *Amphicarpaea monoica* the corolla is present but rudimentary; in quite a number of species the corolla shows lesser degrees of reduction, among which she mentions *Impatiens* sp. but does not state which species. In *Impatiens noli-tangere* the petals appear as whitish scales. (35)

The calyx and corolla do not open, but the two whorls are cast off together as a cap, (Fig. 33) which has been compared in appearance to the calytra of a moss. This phenomenon appears also in *I. noli-tangere* (26) and in *I. pallida*. Bennett (4) and Gray (18) both figure these caps. Bennett suggests that the mechanism of expulsion may be in part the elastic filaments. In the expelled caps the somewhat coiled position of the filaments suggests this condition. The expansion of the ovary would seem to play also an important part in the process. Gray's figures suggest this explanation, and caps may frequently be found adhering to the tips of enlarged ovaries as if forced up simply by the increase in the size of the ovary, A similar expulsion of the united mass of sepals, petals and stamens is described for *I. noli-tangere* (26).

Of some interest is the occurrence of crystals and tannin throughout the flowers and other parts of the plant. The occurrence of raphides and tannin is characteristic of the genus (49). Sections even of very young parts show the presence of an internal secretion. This tested with ferric chloride proved to be tannin. It occurs in single cells, or scattered, or in definite layers, and in vessels formed by the disappearance of end walls of elongated cells. These tannin vessels are frequent in the cortex of stem, petioles and peduncles. In hypodermal layers of stem and leaves and through the mesophyll both isolated cells and continuous layers of cells are found filled with the tannin secretion. It is equally abundant in the floral parts. As soon as the bract can be recognized the hypodermal layer shows every cell filled with tannin. As the sepals and petals arise they show similar hypodermal layers. The hypodermal layer of the wall of the ovary is composed of an enlarged zone of cells each filled with tannin (Fig. 34). As the ovules develop their integuments, similar hypodermal layers appear, and the prominent ridges on the seed have the cells filled with the secretion. Even in the anther, in a circle around the connective these cells are prominent in cross sections. Similarly, enlarged cells densely filled with needle crystals are scattered throughout the stem, leaves and floral parts. These appear in the sepals, in the petals, in the filaments and anther walls, and even in the developing pollen (Fig. 11), also in the walls of the ovary and in the integuments.

IV. The Stamens in the chasmogamous Flower

The stamens in the chasmogamous flower arise as five protuberances in the manner described above. As these protuberances increase in height, they increase in width to broad masses. Before the anther lobes have been differentiated the upper parts of the filaments have become greatly expanded. These expanded portions later come in contact, and form a united whorl of stamens joined in the upper part of the filaments but free below. The adjacent anther walls also fuse to a greater or less extent. From the inner face of each stamen develops a broad flap of tissue which extends inward above the pistil (Fig. 5). These may represent ligules from the microsporophylls. These ligule-like processes grow inward until they meet in the center. In the center and on their edges, they become organically fused forming a roof over the pistil. Generally no opening occurs in this roof. Occasionally, however, the pistil may be found to have forced its way between the ligular flaps and to project slightly above it. Gray (18) figures the parts thus. Generally the roof seems to remain intact until the staminal whorl drops from its position.

The pollen arises in four tracts, and the four pollen sacs remain for some time after the formation of the endothecium. Gradually the intervening tissue between neighboring sacs is broken down, forming the two pollen sacs of the mature anther. Gray states that the two pollen sacs are sometimes confluent at the apex (18). By this time internal layers of the anther wall have disappeared except for crushed tags adhering to the inside of the endothecium. Shortly before dehiscence the hypodermal layer, which is composed of greatly enlarged cells, begins to take on the thickening characteristic of the endothecium. Adjacent to the connective this enlargement of cells and development of thickenings may involve two or three layers of cells. The epidermal cells become somewhat swollen and vesicular. Toward the point of rupture these epidermal cells disappear, and toward the connective they increase to two layers. It is the tension set up between these two tissues which apparently accounts for the rupture. After dehiscence, the epidermal cells appear as shrunken remnants adherent to the outer wall of the endothecium. If not too old these epidermal cells after dehiscence still show a tendency to swell up. Apparently the loss of turgor and collapse of the epidermal cell releases the tension of the endothecium and dehiscence occurs. Loew (30a) (discussed briefly in Engler and Prantl (49) has described the mechanism of dehiscence in *I. Roylei*, Walp. About the time of origin of the endothecium, pairs of pollen chambers of neighboring stamens become joined to form one cavity, through breaking down of intervening walls. A similar appearance occurs in *I. fulva*, but there is no breaking down of walls between stamens, but rather a dehiscence of the external pollen sacs in the lower part of the anther toward each other. In *I. Roylei*, according to Loew, through a drying of the epidermal cells the anther walls press into the pollen chamber and force the pollen upward as a column. Dehiscence in *fulva* occurs longitudinally down the inner face. Usually dehiscence takes place before the opening of the flower, so that when the sepals and petals of the unopened flower are separated the interior is found to contain abundant discharged pollen. Similar early dehiscence occurs in other species (Knuth 26). Several hours to a day after the opening of the flower the whole whorl of stamens becomes detached together and falls from the flower. As the whorl falls it generally carries with it much good pollen. In experiments with self-pollination noted above on *I. fulva*, *pallida* and *sultani* the pollen from the fallen anthers was used to pollinate the stigma with entire success. Bennett[4] states that he never found that the stamens fell away spontaneously, but that the whole flower fell carrying the still concealed pistil with it. This conduct of the plant in England seems to have been an abnormality, perhaps induced by a somewhat cold, moist, unfavorable climate in a foreign home. In America the petals and sepals persist for several hours or a day after the fall of the stamens; flowers bearing pistil but no stamens are normal in *fulva* and *pallida* and in *sultani* in the greenhouse.

In view of the structure of the staminal whorl, the occurrence of spontaneous self-pollination in the experiments with *pallida* and *fulva* described above, and the occurrence of pseudocleistogamy present a problem. The ligule-like staminal processes roof over the pistil in such manner that no pollen from the anthers could lodge upon the stigma. In the experiments referred to, the flowers before opening were enclosed in paper envelopes so as to exclude insect visitors. A number of these flowers set seed. In the pseudocleistogamous flowers fertilization occurred before the opening of buds that morphologically were of the chasmogamous type. Fertilization occurred at various stages from that when the bud was five millimeters in length to the mature flower just about to open its sepals and petals. There are three possible methods by which pollination might occur before opening of the flowers. In the first place, the pistil might, as Gray figures, protrude through the staminal ligules and pollen grains lodge upon it. The pistil is figured protruding through the staminal ligules in *I. balsamina* by Engler and Prantl (49) and in *I. noli-tangere* by Le Maout and Descaisne (27). Knuth (26) describes the protrusion of the pistil in *I. noli-tangere*, *parviflora* and *balsamina*. In *I. latifolia*, the stigmas are not covered (30a). The detection of this protrusion in pseudocleistogamous flowers would be a little difficult owing to the fact that the condition of pseudocleistogamylis not likely to be recognized until the perianth cap is being pushed off. At this stage the stamens are so far shrivelled and dried as to place little value upon any appearance of opening through the staminal flaps. Furthermore, the protrusion of the pistil in this manner is of apparently rare occurrence in the chasmogamous flowers, yet the percentage of spontaneously self-fertilized flowers in *fulva* was surprisingly high, three out of five cases.

The second possible method by which self-pollination could occur is by the growth of pollen tubes directly through the mass of the staminal roof to the stigmatic sufaces below. No pollen tubes taking this course were ever detected either in the dissection of the flowers or in microscopic sections. Such negative evidence is, however, of little value because a structure as delicate as a pollen tube would hardly remain intact through the drying and shrivelling of the tissue that occurs before these pseudocleistogamous flowers are recognized.

A third possibility is the detachment and dislocation of the staminal whorl before the opening of the flower. In the smaller pseudocleistogamous flowers this dislocation would be impossible because of the small size of the flower and the compact condition of the floral parts. In the older stages there is a possibility that it might occur. The failure to detect it cannot be taken as evidence against the existence of this condition, as no definite search was made with its detection in mind. Further investigation is necessary to explain the method of self-pollination in these cases.

The arrangement and behavior of the stamens in other members of the genus do not always agree with *fulva*. In *pallida*, the staminal structure cannot be distinguished from that of *fulva*. In *sultani* the structure is similar, but the relative sizes slightly different. In the complete covering of the pistil by the ligular flaps, in the dehiscence of the anthers before opening of the flower and in the subsequent fall of the whole staminal whorl, *sultani* resembles *fulva* and *pallida*. In *noli-tangere*, (Knuth 26, II p. 236) dehiscence occurs before opening of the flower, subsequently the anthers separate and the stigma matures. In *balsamina* (Knuth 26, II p. 236) the mechanism is the same as in *noli-tangere*. Knuth reports the mechanism of *parviflora* as being similar to that of *balsamina*, but in many of these forms the whorl of stamens is easily detached and would readily fall away owing to the resupinate position of the flowers.

The Stamens in the cleistogamous Flowers

The early stages of staminal development in the cleistogamous flowers are exactly like those of the chasmogamous, but the mature condition is strikingly different. Not infrequently one or two of the stamens are shorter than the others. The mature stamen is narrow and strap shaped. There is no tendency to broaden and unite with adjacent stamens as in the chasmogamous. The flap-like ligular appendages on the inner face are absent. The stamens meet above the pistil, but there is no tendency toward fusion. Bennett has published good figures of these in their mature condition (4 fig. 10 and 11). The pollen sacs are greatly reduced in capacity and only two, instead of four as in the chasmogamous flowers (Fig. 15). Each sac is surrounded by an endothecium which is exactly similar to that of the chasmogamous anthers. The great extent of the endothecium in the cleistogamous flower is striking. Relatively it is much greater in extent than in the chasmogamous flower, being well developed on the connective side of the pollen sac, as well as on the outer sides. The endothecium possesses similar thickenings and opens by a stomium on its inner face (fig. 15). The quality of pollen is very greatly reduced, frequently only two or three grains appearing in a microscopic section, and these nearly filling the diminutive pollen sac. The anthers dehisce (Fig. 33) but the pollen grains are not discharged from the pollen sacs. They germinate within the sac and the pollen tubes pass through the stomium directly down to the stigmatic surfaces below. Thus, although there is a great reduction of pollen, there is also a great reduction in the amount of waste.

This reduction of parts in the anthers is common to cleistogamous flowers. In *I. noli-tangere* the pollen sacs are small, not containing more than forty or fifty pollen grains. An endothecium is present and dehiscence occurs, but the grains germinate in the pollen sacs and the pollen tubes pass out to the stigma below (26 p. 53). In *Oxalis acetosella* "the number of pollen grains in each loculus may not exceed two dozen" (26). Helene Ritzerow (41) gives a summary of conditions in various cleistogamous flowers, listing species in which the various conditions occur. In not a few species a reduction in the number of stamens occurs. Perhaps the occasional occurrence of one or two shorter stamens in *fulva* is a tendency in this direction. In the majority of species there is a tendency to reduction in the number of pollen sacs from four to two. An endothecium is present in all cases except in *Amphicarpaea.* In the majority of species, the pollen grains germinate in the anthers; in a few they fall out and germinate on the pistil. The pollen tube may pass directly through the wall if the endothecium is absent or does not open, if it opens they pass through the opening.

Development of the Pollen

The development of the pollen is the same in each type of flower. The archesporial cells were first recognized when separated by one parietal layer, from the epidermis (Fig. 10). One or two cells appeared thus in the cross sections of the young anther lobes. In longitudinal sections these appeared as a plate of cells one layer in thickness. The primary parietal layers divide to form four or five layers. The outermost parietal layer, immediately under the epidermis, becomes differentiated as the endothecium. The tapetum arises as a single layer from the sporogenous cells. This primary tapetal layer later becomes a double tapetal layer (Figs. 11 and 12). By the time the sporogenous cells have arrived at the mother-cell stage the tapetum shows signs of flattening as if through crushing by the sporogenous mass, or by breaking down to furnish nourishment for the developing spores. By the time the tetrad stage is reached the tapetum and one, two, or three parietal layers have collapsed (Fig. 12). Before the mother-cell stage is reached a remarkable degeneration of potentially sporogenous tissue is proceeding (Fig. 11). Plates of cells ramifying through the sporogenous mass undergo this process of degeneration. Among these plates of cells appear cells filled with needle crystals (Fig. 11). Similar needle crystals are quite common in the wall and other parts of the different members of the flower. This degeneration of pollen is generally quite extensive, involving at times fifty percent of the potentially sporogenous tissue. The history of the pollen is an illustration of the great amount of loss suffered by originally sporogenous cells. First, the early cutting off the tapetum removes a large percentage of available tissue, then the extensive degeneration of sporogenous cells, and lastly the loss mentioned above of a very considerable amount of pollen with the falling away of the whorl of stamens before the total discharge of pollen. In the cleistogamous anthers the loss through the organization of the tapetum involves as great a per cent as in the chasmogamous anthers; the sterilization through degeneration is less extensive, however, and as the pollen grains have germinated before the fall of the cap, practically none is lost at this stage.

At maturity the pollen grains are identical in the two types of flowers. They are oval or somewhat barrel-shaped measuring on an average 0.03 × 0.019 mm. The intine and exine are quite evident in the one nucleate pollen grain. The exine in both types of flowers becomes marked with flat-topped ridges dividing the surface into more or less hexagonal areas. In the chasmogamous flowers the pollen grains are sometimes loosely held together by threads. In the cleistogamous flowers these threads do not appear; but if their function is to hold the grains together, they would be functionless in the cleistogamous anthers where the amount of pollen is very small, and it is never discharged from the pollen sacs. There are easily distinguished four points of exit of the pollen tubes. When germinated in sugar solution four tubes issue from these points and attain a considerable growth. It was not discovered whether the pollen grains on the chasmogamous stigma gave forth more than one tube apiece, but in the germinating grains of the cleistogamous anthers only one tube issued from a grain in the cases examined.

The germination of the microspore is quite typical. The one nucleate stage is of relatively long duration (Fig. 13). The spore coats become differentiated and the exine marked with the characteristic ridges. Division gives rise to a large tube nucleus and a smaller generative nucleus. The tube nucleus is round and takes a position in the middle of the sac. The generative nucleus takes a position alongside and becomes much elongated (Fig. 14).

V. The Pistil

The early stages in the development of the pistil were referred to briefly above. Three small prominences mark the origin of the pistil (Fig. 5 A). These rise separately for a time, then the tissue rises as a ring with more or less of a gap on one side and bearing the three prominences on the top. The septa arise as five prominences pushing in from the ovarian walls (Fig. 6). These prominences meet the rising placenta and so divide the ovary into five chambers. Before the ovary has closed over, one ovule generally has arisen in each chamber. These arise laterally from the central placenta. This history suggests the cauline nature of the ovules. Generally one ovule only arises in each cavity, and five seeds is a common number in the mature capsule. Before the ovule has reached the fertilization stage the septa show signs of breaking down in their internal layers of cells (Fig. 34). This degeneration continues in later stages.

At pollination the ovary is five-celled. The style is practically absent. The stigmatic surface covers the upper and inner faces of five little teeth on the top of the ovary. Near the bases of these stigmatic teeth open the stylar canals. The stylar canals originate by the failure of the ovary to close over completely in its upward stylar prolongation (Fig. 33).

By the time the archesporial cell is clearly recognizable the inner integument can be made out as a prominence on the side (Fig. 16). Before the mother-cell has reached the synapsis stage, the inner integument has reached the top of the nucellus and the second integument is indicated (Fig. 24). As in other anatropous ovules, the second integument is absent on the funicular side of the ovule. The second integument continues growth until it approaches the inner integument in height. This condition is attained before the mother-cell has divided. From this point onward the two integuments rise together. Hence only at the tip are the two integuments to be distinguished. Below the tip they are indistinguishable under the microscope. They are not fused in the sense that two separate tissues have fused, but the tissue below the tip arises as one continuous tissue. The layer of the integument lying against the nucellus early becomes differentiated as a zone of short broad cells apparently tapetal in function. This jacket layer is remarkably persistent through the history of the ovule. It remains after the destruction of the peripheral nucellar layer and consequent increase of the size of the embryo sac after fertilization. (Fig. 28) After the embryo has filled the embryo sac, this layer appears as a densely staining but somewhat crushed investment (Fig. 35). Miss Riatt (39) reports a similar jacket layer in the innermost layer of the integument of *pallida*. To judge from her figure 1, H, however, there is one point of difference from *fulva* in the differentiation of the two integuments. The line of sepaiation between the two integuments is shown reaching well down to the basal end of the ovule.

The development of the megaspore and embryo sac presents nothing unusual. The archesporial cell is recognizable as a hypodermal cell soon after the ovular swelling becomes evident (Fig. 16). No parietal cell is cut off; and so the archesporial cell becomes the megaspore mother-cell. The megaspore mother-cell divides into two (Fig. 17), and then four (Fig. 18), forming a linear tetrad of megaspores. The innermost megaspore of the tetrad rapidly enlarges and the others rapidly degenerate. By the time of the first division of the megaspore nucleus the outer megaspores appear as crushed degenerating masses (Fig. 19). Divisions into four and eight nuclei follow quickly (Figs. 20 and 21). Three antipodal cells are cut off and rapidly degenerate (Fig. 22). The two synergid nuclei move to positions side by side in the upper end of the sac. A vertical wall separates the two, then horizontal walls cut them off as separate cells. (Figs 22 and 23). They rapidly degenerate and, at the time of entrance of the pollen tube, appear only as crushed remnants. The two polar nuclei move to the center of the sac and lie in contact, closely appressed, until fertlization (Figs. 22, 25, and 26). The egg lies in position immediately above the polars. Miss Riatt (39) states that no antipodals were found in *Impatiens pallida*. She states that the synergids lie below the egg toward the center of the sac. In her figure at the time of fertilization, when she says that no polar nuclei are shown, the structures which she marks synergids resemble the polars of *fulva*.

There are some interesting points in the history of the nucellus. The archesporial cell arises as a hypodermal cell. No parietal tissue arises, so that never more than one layer of nucellar tissue overlies the embryo sac. By the first division of the megaspore the nucellar cells overlying the apex may show signs of shrinkage (Fig. 17). At the stage of fertilization the cells over the apex are disappearing, and cells along the side of the embryo sac, never more than one layer in thickness, show signs of disintegration. The cells below the embryo sac, generally three layers, at an early stage become elongated and tracheid-like (Fig. 17). After fertilization the sac begins a rapid elongation, crushing the tracheid-like cells below, and apparently absorbing their contents for its growth. The elongation continues to the bottom of the ovule, then turns and grows a short distance in to the funiculus. At its greatest development, the embryo sac is thus a long narrow structure sharply curved at its lower end (Fig. 36).

The conditions in *I. pallida* seem to be in some points exactly the same as in *fulva*. To judge from Miss Riatt's figures 1, J and K, and 2 L, the nucellus surrounding the embryo sac is one layer in thickness. The cells below the sac are in three rows, which with the extension downward of the lateral layers above make five rows. The cells show the same elongated tracheidal form, and like *fulva* extend downward and curve into the funiculus. The degeneration of the peripheral layer begins apparently in the megaspore stage (Riatt, fig. 1 J). In the fertilization stage the nucellus is apparently entirely gone at the sides and micropylar end, and the embryo sac is against the jacket layer (Riatt Figs. 2 O and S).

The cleistogamous pistil is similar in development and structure except for a certain reduction. It arises in the same manner at first as three papillae and later as a ring of tissue. The septa advance from the side walls to meet the central placenta, dividing the ovary into five chambers. In the pseudocleistogamous flowers the five little teeth surmounting the very short style are much reduced or even absent, and in the cleistogamous flowers they are generally absent entirely. The stylar canals are present as in the chasmogamous flowers. In the pseudocleistogamous flowers the full number of ovules may be present, but in the cleistogamous flowers these are reduced to three, two or one. The reduction of the number of seeds in the cleistogamous capsules is not due to failure of fertilization or subsequent degeneration, but to a reduction in the number of ovules which arise. The archesporial cell divides to form four megaspores, and the chalazal spore persists. Some sections show only three megaspores apparently, through failure of one daughter cell to divide; often, however, the four megaspores appeared in a linear series. The embryo sac forms eight nuclei, and the antipodals and synergids are cut off as in the chasmogamous flowers. The mature sac contains the egg and below it the two polar nuclei. The history of the nucellus and integuments is the same as in the chasmogamous ovule.

As in other cleistogamous species, the pistil of the cleistogamous flower differs from that of the chasmogamous chiefly by reduction of parts. In *I. fulva* the reduction affects the short style and stigma and the number of ovules. In *I noli-tangere* five short styles persist. The reduction of style and stigma is common in cleistogamous flowers. Helene Ritzerow (41) lists some ten species where it occurs. In *Aspicarpa hirtella*, *Aspicarpa longipes* and in *Specularia* a reduction in the number of carpels occurs (41, p. 212). On the whole, however, the pistil seems to suffer less reduction than do other parts of the flower in cleistogamy.

VI. Growth of Pollen Tubes and Fertilization

On germinating the grains in sugar solution, four tubes begin growth from the four corners of the somewhat barrel-shaped or elliptic pollen grain. The behavior of the nuclei with respect to these four tubes was not discovered. In the few cases examined of pollen grains germinated on the chasmogamous stigma, each grain gave forth apparently only one pollen tube. In the cleistogamous flowers, the development of the pollen tube from the grain is more easily followed. Here only one tube issued from each grain. The tubes pass directly down from the cleistogamous anthers to the opening of the stylar canals (Fig. 33). Through these open canals the tubes pass in such numbers as to fill them. Issuing from the lower end of the short stylar canals, the tubes pass in fairly direct course to the ovules, where they may be seen in numbers clustered about the micropyle. The inner integument about the micropyle at this stage takes a vivid stain, suggesting the presence of some substance attractive to the pollen tubes.

The history of the tubes and their nuclei in the embryo sac is the same in both chasmogamous and cleistogamous flowers. As the pollen tube issues from the inner end of the micropyle, it appears in two or sometimes three branches (Figs. 25 and 26). Miss Riatt (39) reports similar branches in *Impatiens pallida*, and it is recorded for other genera. As these branches pass down through the embryo sac, one nucleus in each branch becomes much larger (Figs. 25 and 26). This is the functioning male nucleus. In addition to this nucleus, there appear one or two smaller degenerate looking masses of nuclear material. These are apparently the remains of the degenerating tube nuclei. Finally they break into fragments and are lost. One branch of the tube approaches the egg, and the other approaches the two polar nuclei. The polars are now closely appressed, but entirely distinct. The ends of the tubes swell out and rupture, and the male nuclei pass out one to the egg and one to the polars.

VII. The Growth of the Embryo

After fertilization, the primary endosperm divides before the fertilized egg. By the first division of the fertilized egg the endosperm nucleus has divided to form several nuclei. The endosperm nuclei are much smaller in diameter than the egg nucleus. They become arranged around the edge of the sac, and move upward around between the egg and the micropyle (Fig. 27). The first division of the egg is transverse (Fig. 27). The upper of these cells develops the suspensor, and the lower the embryo. The upper cell divides transversely and the lower one longitudinally. In Figure 28 three cells of a four-celled embryo appear. The two upper cells are derived by the division of the cell which gives rise to the suspensor. The lower cell of Figure 27 has divided in the plane of the paper, one of the daughter cells appearing in Figure 28, and one lying immediately behind appearing in the next section on the slide. The next division is longitudinal and at right angles to the last, giving rise to the quadrant stage of the embryo. Figure 29 is a section through the embryo at the stage when the dermatogen is cut off. The section is not central, so that only one of the central cells appears. The outer layer of dermatogen cells is evident. The suspensor at this stage is at first two cells in length (Fig. 29). This, however, rapidly increases to five cells in length (Fig. 30). In the meantime, the lower two of the suspensor cells have divided longitudinally (Fig. 31). Further division gives rise to two layers of several cells. Then the third cell of the suspensor divides longitudinally to form a plate of at first four cells. Meanwhile the divisions of the cells of the embryo have continued. The plerome and periblem are differentiated. Toward the micropylar end of the embryo these layers do not arise from the original embryo cell. The three lower layers of the suspensor continue to divide and form the periblem and dermatogen in this region. The calyptrogen is later added from the outermost of these layers. The two outermost cells of the suspensor remain undivided (Figs. 31 and 32). These elongate, push into the micropyle, and form the suspensor proper. Figure 32 shows the embryo at the time of origin of the cotyledons.

A peculiarity of the seeds of the genus is the existence of four side roots already developed before germination. Heinricher[21] reports this condition in a number of the Graminaceae and on Goebel's authority in the genus *Cucurbita*. Heinricher found it in all species of *Impatiens* that he investigated:—*I. glanduligera*, Royle, *I. scabrida*, D. C., *I. noli-tangere*, L., *I. parviflora*, D. C., *I. bicornuta*, Wall. and *I. balsamina*,

L. These four roots form a whorl, one pair falling in the median plane of the cotyledons and one at right angles. In cross section these appear as the arms of a cross. On germination these appear soon after the primary roots as little hooks and soon press into the ground. Heinricher's figures show the condition in *I. glanduligera* and *balsamina*. Heinricher states on Klebs' authority that in *Impatiens noli-tangere* at germination the four side roots surpass the main root. Similar behavior of side roots is seen in *fulva*. The side roots were not found, however, already developed in the seed.

The reserve food stored in the cells of the cotyledons has been investigated in several species of *Impatiens*, particularly in *I. balsamina* by Heinricher. In *I. balsamina*, *capensis* and others reserve food is stored in the form of cell-wall thickenings. These thickenings Heinricher (21) determined were not cellulose but of amyloid nature. The thickenings disappear on germination, and starch grains appear in the cells. Subsequently the cells become green and function as assimilative cells. The cells of the cotyledons of other species of *Impatiens* contain small protein grains and a large quantity of fatty oil (21, p. 167) with no carbohydrate. In *I. fulva*, the cells of the cotyledons are thin-walled, and densely packed with large grains of reserve food. This material proved not to be starch, but its nature was not investigated. It may be the fatty oil characteristic of other species of the genus.

The history of the endosperm presents some interesting points. The primary endosperm nucleus divides quickly and divisions follow in rapid succession. The nuclei move to the periphery of the embryo sac. Some push around above the embryo and even more into the micropyle (Fig. 27). The nuclei in the micropylar end of the sac become cut off by cell walls (Figs. 28 and 29). These cells soon begin to show signs of degeneration, and in later stages appear as small crushed masses in the micropyle and in the micropylar end of the embryo sac (Fig. 30). The nuclei on each side of the embryo a little later are cut off as cells. Thus when the cotyledons begin to appear three rather irregular layers of endosperm cells are to be seen at the sides of the embryo (Fig. 32). As the embryo increases in size the peripheral layer of endosperm nuclei elsewhere in the sac become cut off into cells. At the micropylar end of the sac the cells become much elongated, forming a row around the hypocotylar end of the embryo as if functioning as a suspensor to force the embryo down toward the center of the sac. There seems to be quite a tendency for cells to force their way to the micropylar end of the sac or even into the micropyle. The synergids when cut off become more or less rounded and push up into the lower end of the micropyle and there degenerate. Then the first endosperm nuclei that are cut off move around the embryo and take positions close to and in the micropyle where one or two endosperm cells may be seen cut off (Figs. 29 and 30). These sooner or later degenerate. Then these suspensor-like endosperm cells arise at the micropylar end and persist for a considerable time. The peripheral layer increases to a double layer of cells lining the embryo sac. When the embryo has completely filled the sac and the outermost layer of the integument is beginning to take on the peculiar differentiation of the seed coat, one of these layers may be still made out. It disappears, however, before the ripening of the seed.

VIII. Development of Seed Coat and Pericarp

The behavior of the seed coats after fertilization is worthy of note. At the stage of fertilization the outer integument has risen almost to the level of the inner integument. The two integuments are distinguishable only for a short distance at the micropylar end. Below this point the tissue is perfectly continuous; no dividing line can be found between the two. This condition results from the growth of the lower part not as two separate integuments that afterwards fuse, but as one tissue that rises as a whole. The inner layer of the integument lying next to the nucellus, and, after the destruction of the nucellus, against the embryo sac, is composed of tabular cells forming a distinct jacket layer (Fig. 28). Very soon after fertilization, degeneration begins in the region about three layers below the jacket cells (Fig. 36). This degeneration spreads inward toward the jacket layer and outward toward the exterior. The jacket layer becomes shrunken to a thin densely-staining layer surrounding the embryo (Fig. 35). The degeneration of tissue continues outward until only two or three layers remain intact. Meanwhile at the micropylar end the outer integument has grown beyond the inner (Fig. 36). The cells of the outermost layer of the integument become much enlarged and extend outward to form the characteristic ridges of the mature seed coat (Figs. 34, 35 and 36). The cells gradually become filled with the tannin that is so characteristic of the genus.

The early history of the ovary has been considered above, the development subsequent to fertilization remains to be considered. The septa have begun to show signs of degeneration in their inner layers before division of the megaspore mother-cell (Fig. 34). This degeneration continues rapidly until at fertilization the septa consist each of the two outer walls alone. By the time the cotyledons have been differentiated the septa are represented only by tags adhering to the placenta and ovarian wall (Fig. 35).

Early in the history of the ovary, very soon after its appearance and before the appearance of the ovule, there is a striking differentiation in the hypodermal layer as in other members of the flower (Fig. 34). This layer is composed of large, regular, squarish cells quite distinct in shape and size from neighboring layers. The cells very early become filled with tannin which adds to their prominence in a section. At five points, opposite the centers of the septa, the large squarish cells are replaced by two, or sometimes three or four small cells also filled with tannin (Fig. 34). Below these small hypodermal cells a line of small cells extends transversely through the ovarian wall to the inside. These mark the lines of rupture. Some time after the total degeneration of the septa, the innermost layer of the ovarian wall begins to show signs of degeneration and shrinkage. This degeneration extends up the small lines of cells that mark the lines of dehiscence. As growth continues degeneration extends backward until it reaches the vascular bundle in the middle of the valve and until it reaches the hypodermal layer in the lines of dehiscence (Fig. 34). The epidermal cells by this time have become filled with tannin, and are in a state of turgescence. No mechanical thickenings appear. The bursting of the pod is controlled apparently by turgescence of the hypodermal and epidermal layers.

Conclusions as to Cleistogamy

Many of the attempts to explain the phenomenon of cleistogamy have been based on teleological considerations. Knuth, (26) for example, explains the appearance of cleistogamous flowers in *Drosera rotundifolia* by the assertion that insects are more attracted by the glistening drops on the glandular leaves and do not visit the flowers. "Owing to the continual capture of insect-prey open flowers are useless for the sundew, and it therefore develops cleistogamous ones." In *Oxalis acetosella*, the cleistogamous flowers are developed in June and July because insects would not visit the rather inconspicuous flowers of *Oxalis* when more showy flowers are in blooom, but in spring when the plant does not have to compete with more showy forms the chasmogamous flowers appear. Knuth explains the occurrence of cleistogamous flowers in the *Anonaceae* on the authority of Loew as an adaptation for protection against ants. Darwin (13, p. 338) found the explanation in "the production of a large supply of seeds with little consumption of nutrient matter or expenditure of vital force." Delpino explains cleistogamous flowers as an adaptation to secure the production of seed under favorable climatic conditions. Shaw, (45) after giving an historical résumé of the subject, places emphasis on the cleistogamy as an adaptation to secure the rapid development of fruit.

The explanation of cleistogamous flowers as stages of arrested development has been offered by various authors since the phenomenon has been an object of investigation. Gray, (18) as long ago as 1849, regarded the cleistogamous flowers of *Impatiens fulva* as buds arrested at an early stage. Darwin agrees in part with the arrested development idea, but offers some objections to it which argue for his view noted above. Since Darwin's time, the conditions which bring about the arrest have been determined through a wide range of observation and experiment by various investigators. Temperature, toxicity of soil attacks of parasites, reduction of leafage, moisture and light have been demonstrated to be associated with the production of cleistagamous flowers. Goebel (17) finds that all of these conditions affect the production of cleistogamous flowers through the unfavorable conditions of nourishment. He regards the cleistogamous flowers as "Hemmungsbildungen" of the chasmogamous flowers to be explained solely on the ground of nourishment. The occurrence of the arrest in all stages adds weight to the view. From the smallest cleistogamous flower with no resemblance to the evolved zygomorphic chasmogamous type, gradations may be shown through various sizes and degrees of pseudo-cleistogamous flowers to the type which Hansgirg (20) originally gave the name pseudo-cleistogamous, a flower similar in every respect to the chasmogamous flower, but failing to open before fertilization. It is but a step more to include in the series those showy flowers which normally are self-fertilized without opening or even to the expanded self-fertilized species.

Burck (11) added a new thought to the investigation when he ascribed the origin of cleistogamy to mutation. It is worth noting, with mutation in mind, the variety of families in which cleistogamy is reported. The list of families given by Knuth (26) in which cleistogamy occurs, shows a range from some of the simplest to the most evolved flowers. Among the Monocotyledons it occurs in the relatively simple Alismaceae, Butomaceae, Graminaceae, Pontederiaceae; in the more evolved Juncaceae and Liliaceae; in the most evolved family, the Orchidaceae. Among Dicotyledons, it occurs in the less evolved Caryophyllaceae and Portulacaceae; in the more evolved Rosaceae and Oxalidaceae; and finally in the Labiatae and Scrophulariaceae. Families lying between the extremes show abundant occurrence of cleistogamy. It would seem that if mutation is the sole cause of cleistogamy, it must have arisen many times in these widely separated families. This multiple origin, however, would not necessarily be an objection to the theory, as there is abundant evidence of other plant structures appearing in divergent groups of plants.

The evidence at hand is on the whole in support of Goebel's explanation of cleistogamous flowers as a product of reduced nourishment. Darwin thought the existence in cleistogamous flowers of altered structures especially adapted for cleistogamous fertilization argued against the idea of arrested development (12). He mentions in particular the altered style in Viola and the lack of style and the open stylar canals in many forms. Bennett believes the difference between the two types of flowers is original, but the grounds for his opinion are not safe. He described a difference between the sepals of cleistogamous and chasmogamous flowers in the earliest recognizable bud. This, however, is a comparatively late stage. The arrest may be effected in the primordia of the flower, or perhaps the "arrest" or alteration in vital processes may take place at even an earlier stage, before the primordia have appeared at all. When alteration of structure does occur in the cleistogamous flowers it is generally a matter of the reduction of parts. The petals fail to develop or develop only to a reduced size, the stamens lack parts as in *Impatiens*, or their number is reduced even to one stamen, the style is lacking and stylar canals remain open, the number of ovules is reduced. These are plainly differences due to loss or reduction of parts. Where deviation occurs in the line of apparently added structures, these structures are evidently due to such growth as occurs from altered primordia. The conditions which cause these deviations have been determined; the morphology of the mature parts has been described in a great variety of forms; some work has been done on the histological developments of parts. Further exact work is needed to determine the point at which histological differentiation first occurs, and to determine the exact physiological processes which result in histological deviation in the two types of flowers. When there is more information along these lines, further attempts to explain the biological significance of cleistogamy will be in order.

Technique

The method of handling experimental materials was described above in reporting the experiments. For histological work, material was fixed in chrom-acetic, Flemming's solution, and Davis's modification of Renner's solution. The latter solution consists of:—

90% alcohol	300 cc
distilled water	200 cc
nitric acid	10 cc
bichloride of mercury	to saturation
glacial acetic	5 cc

This gives a 60% solution which for use is diluted to 40% by the addition of water. The material is left in the solution from five to seven hours. For embryo-sac and other delicate structures, the shorter time is best. For embryo-sac and earlier stages of pollen development, this solution proved superior to chromacetic, and for the histology of the structures quite as good as the expensive Flemming solution. The material was stained in Haidenhain's iron-alum haematoxylin, and in safranin and gentian violet combination. Counterstaining the iron-alum-haematoxylin with safranin or orange G proved advantageous in some cases.

IX. SUMMARY

I. Plants of the species at maturity vary in height from eight inches to seven feet. Adverse conditions of dryness or toxicity of soil bring about dwarfing. Optimum conditions for growth are abundant water with good drainage and light shade or sun.

II. Chasmogamous flowers appear in late June and last till early October.

III. Cleistogamous flowers appear in early June and last through the summer. They are most abundant before the chasmogamous flowers appear. They are to be found throughout the summer on plants under adverse conditions, and on the lower, short side branches of many plants under good conditions.

IV. Pseudocleistogamous flowers are morphologically *chasmogamous buds* which are self-fertilized at various stages, from the size of four millimeters to a mature flower about to open.

V. Humming-birds and bees are the agents of pollination. The pollen is shed upon the bee as the insect forces its way to the nectary. Later the whorl of stamens falls away and the stigma is exposed.

VI. The flowers are readily self- and cross-fertilized, and seed was produced by cross-pollination between *I. fulva* and *I. pallida.*

VII. The size of cleistogamous and chasmogamous seed is the same. The number in chasmogamous capsules is four or five; in cleistogamous capsules one to three.

VIII. Drought, weak light, poor soil, excessive temperatures, toxicity of soil and reduction of leafage inhibit chasmogamous flowers, but cleistogamous flowers may be produced under these conditions.

IX. There are two views as to the morphology of sepals and petals. According to the first view, there are three sepals and three petals, two of the petals being formed by the fusion of four in pairs. According to the second view, there are four sepals and two lobed petals.

X. The cleistogamous flower shows all the members that appear in the chasmogamous flowers. The petals and sepals are forced off by the expanding ovary as a cap, resembling in appearance the calyptra of a moss.

XI. The chasmogamous stamens are coherent, with broad filaments and with ligular appendages which are coherent above the pistil; the cleistogamous stamens are separate, strapshaped, without appendages.

XII. The development of the pollen is peculiar in the degeneration of plates of sporogenous cells.

XIII. The cleistogamous stamens have a very small number of pollen grains. An endothecium is formed and dehiscence occurs, but the pollen grains remain in the pollen sacs and send down tubes to the stigma.

XIV. The nucellus is a single layer of ephemeral cells at the sides and micropylar end; below the embryo sac it is composed of five rows of elongate tracheid-like cells which disappear as the embryo sac enlarges. The mature embryo sac is a long curved structure.

XV. There are four megaspores, the inner one becoming the embryo-sac.

XVI. Eight nuclei arise in the embryo sac; three are cut off as antipodal cells, and two as synergids. Antipodals and synergids are ephemeral.

XVII. The pollen tube branches on entering the embryo-sac, each branch carrying a male nucleus.

XVIII. The first division of the fertilized egg is transverse, the micropylar cell gives rise to the suspensor, and the lower cell to the major part of the embryo.

XIX. The original suspensor cell divides to form five cells in a line, the lower three subdivide to form the lower part of the embryo, the two micropylar cells elongate to form the suspensor proper.

XX. The free nucleate stage of the endosperm is followed by a mass of ephemeral cells at the micropylar end of the sac; elsewhere the endosperm is a double peripheral layer of cells which disappear at the maturity of the seed.

XXI. The ovule is surrounded by one mass of tissue which only at the micropylar end is divided into two integuments.

XXII. After fertilization, degeneration begins in the middle layers of the integument and spreads in both directions.

XXIII. The dehiscence of the capsule is brought about by the increase in size and turgor of hypodermal and epidermal cells, and the separation of the wall along a line of small cells lying originally in line with the septa.

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PLATE LV

FIG. 1. Cross section of bud of chasmogamous flower with only bract developed. ×110.
A—bract.

FIG. 2. Cross section of bud of chasmogamous flower with lateral and posterior sepals. ×125.
A—bract.
B—lateral sepal.
C—posterior sepal.

FIG. 3. Cross section of bud of chasmogamous flower with four sepals. ×125.
A—bract.
B—lateral sepals.
C—posterior sepals.
D—anterior sepal.

FIG. 4. Cross section of bud of chasmogamous flower with stamens arising. ×100.
E—anterior lobe of petal.
F—stamen.

FIG. 5. Longitudinal section of bud of chasmogamous flower. ×30.
A—carpels.
B—ligular appendage of stamen.

FIG. 6. Cross section of bud of chasmogamous flower showing origin of septa of ovary. ×40.

FIG. 7. Longitudinal section of buds of cleistogamous flower. ×50.
A—bract.
B—posterior sepal.

FIG. 8. Longitudinal section of bud of cleistogamous flower showing origin of stamens ×95.
C—stamen.

FIG. 9. Cross section of mature cleistogamous flower. ×40.
A—bract.
B—lateral sepal.
C—posterior sepal.
D—anterior sepal.
E—anterior lobe of petal.
F—posterior lobe of petal.

FIG. 10. Cross section of anther lobe showing pollen archesporium. ×1000.

FIG. 11. Cross section of anther lobe showing degenerating plates of pollen mother cells. ×125.

FIG. 12. Cross section of anther lobe in pollen tetrad stage. ×100.

FIG. 13. One nucleate pollen grain. ×1000.

FIG. 14. Pollen grain with tube and generative nuclei. ×1000.

FIG. 15. Cross section of cleistogamous anther near maturity. ×200.

Plate LVI

Fig. 16. Ovule showing single archesporial cell and beginning of inner integument. ×300.

Fig. 17. Megaspore daughter cells.

Fig. 18. Four megaspores, ×850.

Fig. 19. Embryo-sac two nuclei. Two degenerating megaspores. ×1000.

Fig. 20. Embryo-sac with four nuclei. ×1000.

Fig. 21. Embryo-sac with egg and two polar nuclei; one synergid cut off above and three antipodals below. ×1000.

Fig. 22. Embryo-sac with six nuclei. ×1000.

Fig. 23. Embryo-sac with two polar and egg nuclei; two synergids cut off above. ×1000.

Fig. 24. Origin of the second integument.

Fig. 25. Embryo-sac showing two polar nuclei; branched pollen tube with male nuclei and remains of tube nucleus.

Fig. 26. Embryo-sac with egg and polars; branched pollen tube with male nuclei and remains of tube nucleus.

Fig. 27. First division of fertilized egg; endosperm nuclei. ×290.

Fig. 28. Three cells of a four-celled embryo; beginning of segmentation of endosperm; the jacket layer of the integument. ×400.

Fig. 29. Embryo showing two-celled suspensor, and dermatogen, and the first cells of the endosperm. ×300.

Fig. 30. Embryo with five-celled suspensor; the first cells of the endosperm degenerating. ×300.

Fig. 31. Embryo showing division of the basal suspensor cells. ×450.

Fig. 32. Embryo with the cotyledons arising, mature suspensor and layers of endosperm cells. ×75.

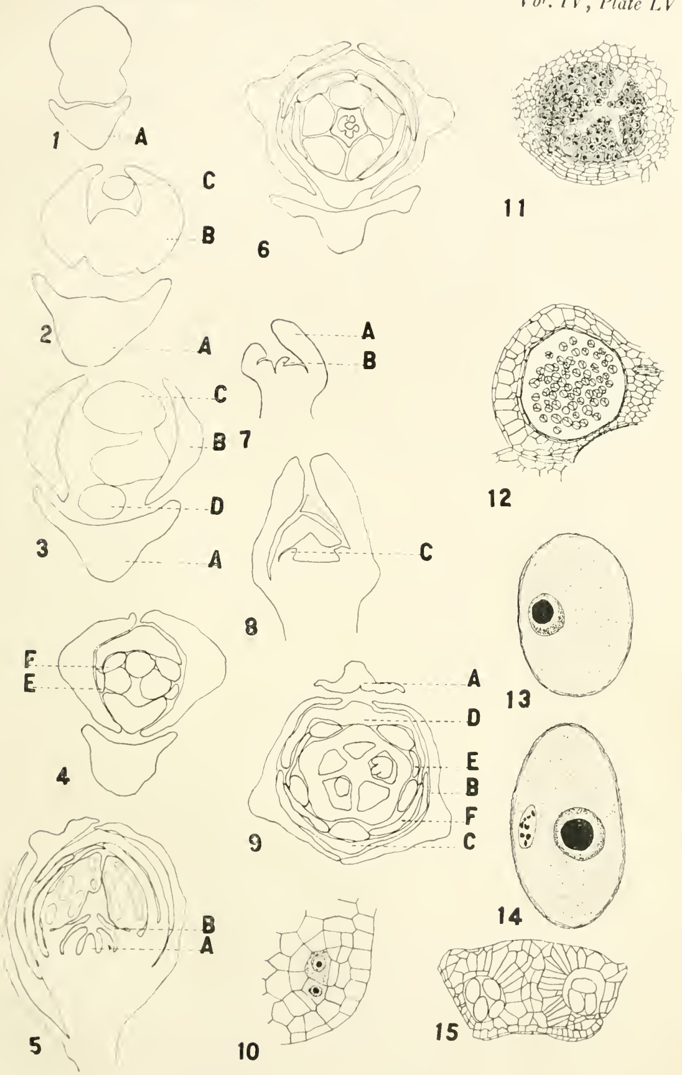
Plate LVII

Fig. 33. Longitudinal section of a cleistogamous flower showing the forcing off of the perianth cap, the pollen tubes and the stylar canals. ×30.

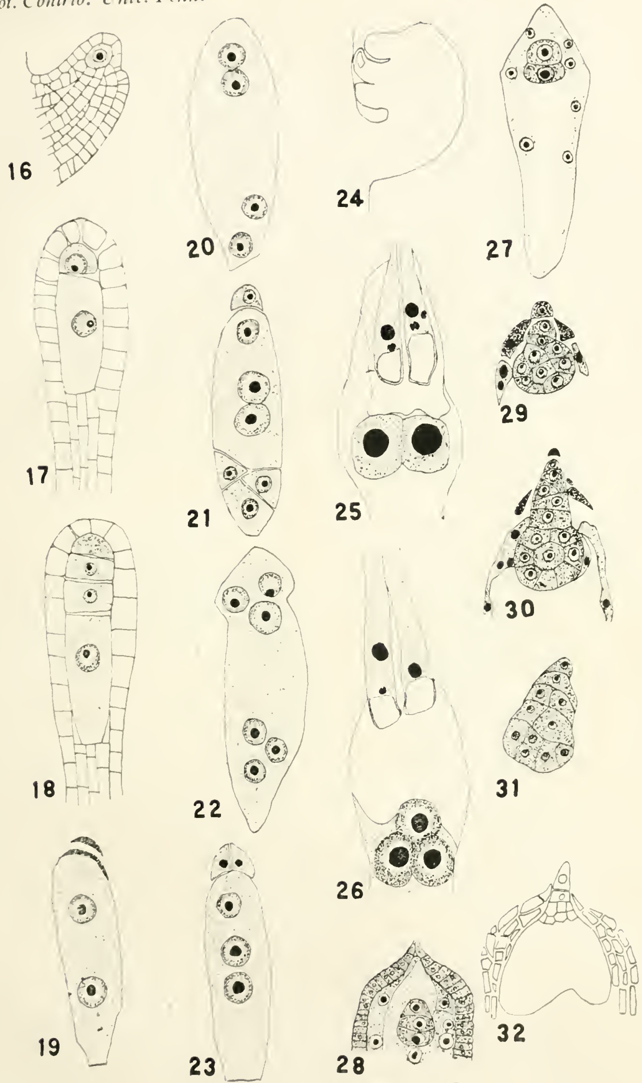
Fig. 34. Cross section of ovary of chasmogamous flower showing the enlarged cells of hypodermal layer, the smaller cells opposite the septa marking the lines of dehiscence and the beginning of degeneration of the septa. ×124.

Fig. 35. Cross section of ovary of chasmogamous flower with seed near maturity showing degeneration of inner layers of ovary wall, the enlargement of epidermal and hypodermal layers of ovary wall, and development of seed coats. ×50.

Fig. 36. Longitudinal section of ovule with embryo showing degeneration of middle layers of integument.



CARROLL ON IMPATIENS



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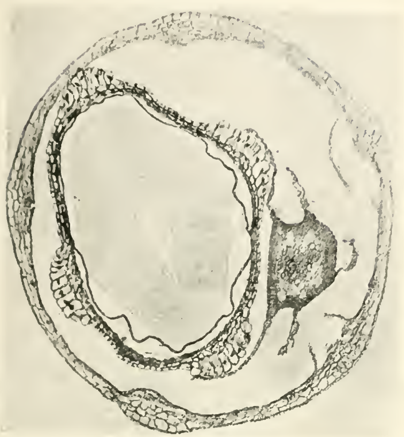

Fig. 35

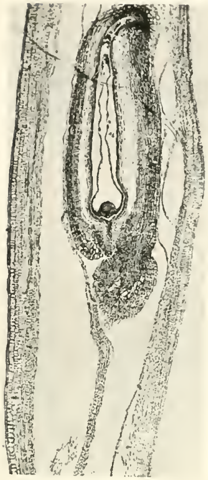

Fig. 36

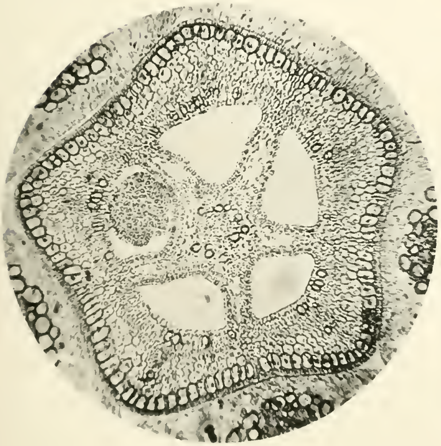

Fig. 34

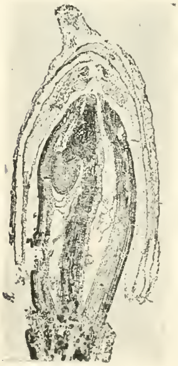

Fig. 33

CARROLL ON IMPATIENS

THE COMPARATIVE HISTOLOGY AND IRRITABILITY OF SENSITIVE PLANTS

BY

D. Walter Steckbeck, M. A., Ph. D.

With plates LVIII—LXV.

Thesis presented to the Faculty of the Graduate School
in partial fulfilment of the requirements for
the Degree of Doctor of Philosophy

INTRODUCTION

The term *Sensitive Plants* was applied by the earlier botanists to certain forms that exhibit the remarkable phenomenon of "closing up on the approach of man, as if they felt or in some way became aware of his approach." The application of the term should not be restricted to comparatively few plants, for all plants—all living organisms—are sensitive in that they respond to and are influenced in a variety of ways by environal factors. The marked difference between "sensitives" and "non-sensitives" lies in the fact that the former have certain mechanisms which enable them to respond relatively quickly to stimuli. Such response usually results in a change of position of the plant or of certain parts of it, and such parts are said to be irrito-contractile. The degree of response, and the duration of the change of position, depend on the character of the stimulus and the time during which it acts.

This investigation is restricted to a study of some of the sensitive plants—those that have the special mechanisms—(pulvini) referred to above. The leaves of these plants are of especial interest from the irrito-contractile relation. A study of these, as well as the stems, was taken up with a view of ascertaining whether any structural details might aid in explaining the sensitivity of these plants, and whether there is a correlation between structural peculiarities and relative sensitivity.

The movements of leaves in response to various stimuli is observed in many plants. This phenomenon has been studied since the time of Pliny. The most sensitive movements, and those earliest observed are the day and night movements. Sensitive leaves at night occupy positions other than those which they occupy by day, exhibiting a closing and an opening movement. Such changes of position are known as

Nyctitropic movements; also called night sleep of plants, since the rising and sinking of leaves and leaflets coincides with the nocturnal sleep of animals and the phenomenon was interpreted earlier in this sense.

Leaf sensitivity as usually understood is almost wholly confined to dicotyledons, although there are several monocotyledons, gymnosperms and pteridophytes that exhibit this peculiarity.

Darwin describes sleep movement of the leaves of one of the water ferns, *Marsilea quadrifoliata*. Each leaf bears four leaflets, each of which is provided with a well-developed pulvinus. During the late afternoon, the leaflets move upward and fold upon each other in such a manner as to form a vertical packet. "When the leaves sleep, the two terminal leaflets rise up, twist half around and come into contact with one another and are afterwards embraced by the two lower leaflets."

Among the monocotyledons, probably the best known example of night turning of leaves is shown by various species of *Maranta*. During the day the rather large blades of the leaves are in an approximately horizontal position, while at night they stand vertically.

Amongst dicotyledons, two families stand out prominently as exhibiting the most specialized types of leaf sensitivity, these the Leguminosae and the Oxalidaceae, two families that in sensitive relations, in structural details, and in distribution show many similarities. Both contain forms that are practically non-sensitive with all transitions from these to the very highly sensitive types. Both have compound leaves in nearly all members, with well-defined primary pulvini at the bases of the petiole, secondary pulvini in some and tertiary pulvini in those with bi-pinnately compound leaves. Both families are essentially tropical in distribution of the sensitive types.

This investigation is confined to the Leguminosae and the Oxalidaceae, for practically all sensitive plants, as the term is usually applied, belong to these two families. Other sensitives, such as Drosera and Dionaea, are omitted. These have been studied very extensively by Darwin, Macfarlane and others.

The work was suggested by Professor John M. Macfarlane, of the University of Pennsylvania, to whom I want to express my deep gratitude for his advice, aid and criticisms.

Historical Review

The phenomenon of leaf sensitivity and leaf movement has proved of botanical interest since the days of the early Greeks. From the time of Pliny, who made studies of such movements, the historical records of leaf sensitivity indicate no definite accounts until the eighteenth century. During this century a number of observations on sensitive movements were published. Among the early observers were Mairan, Bonnet, Linnaeus, Acosta, Alpinus, Ray, Hill, Du Hamel and others. Most of the studies were made on the sensitive plant, *Mimosa pudica*, which had then been introduced into Europe and aroused much interest on account of its leaf movements.

Miller (35) recognized five species of Mimosa, all more or less sensitive. "The first sort (*M. pudica*) is commonly known by the name of Sensitive Plant, to distinguish it from the others, which are generally called Humble Plants, because upon being touched, the pedicel of their leaves falls downward, whereas the leaves of the other sort are only contracted upon the touch."

Hill (20) carefully observed the sequence of motion in *Mimosa pudica* and pointed out that the effect of total darkness on the plant is greater than the rudest touch. He also found that the contact stimulus must be of a sufficient intensity, and that the degree of the subsequent motion depended upon the potency of the stimulus. Hill further observed that the movements of *Mimosa* are less well marked at a lower temperature than that in which the plants have been reared. His explanation for this more sluggish movement is—"This is probably due to the juices stagnating in the clusters of fibres, and to the contraction of the bark by cold." Hill's explanation of the response to the contact stimulus is interesting because it is an illustration of the view current at the time that such motion was due to the fibres which acted like those of muscle. Hill also made observations on the movements of the leaves of *Abrus* and *Tamarindus*, and found that "In these and all others, the degree of elevation or expansion in the lobes, is exactly proportional to the quality of the light, and is solely dependent upon it." Hill's work was severely criticised by his contemporaries.

Lindsay (24) studied especially the fall of the leaf of *Mimosa* when stimulated, and discovered that the force which raises the petiole exists in the lower part of the intumescence (pulvinus), and that which depresses it in the upper. He seems to have considered that the temporary excess of force in either part is produced by an impulsion of the sap from the vessels of the yielding portion unto those of the opposite portion.

Dutrochet (16) concluded that the sleep movements were due to opposite changes of the energy of expansion in the antagonistic halves of the pulvinus. This writer was the first to show that stimuli are conducted through the vascular bundles of *Mimosa pudica*, and concluded that the transmission was due to a pulsation of water. Dutrochet, as well as some of the succeeding observers, attempted to compare propagation in vegetable tissue to that by nerves in animals.

Burnett and Mayo (8) described the structure of the leaves of *Mimosa pudica* and their movements, when stimulated or when the plant enters the condition of night sleep. These observers noted the flush changes which pass over the primary and secondary pulvini when the leaves are stimulated. They believed that the under half of the primary pulvinus of *Mimosa* is more irritable than the upper.

De Candolle (13, p. 647) showed that in many cases even the cotyledons of sensitive plants are irritable, as illustrated in *Mimosa pudica.*

Meyen (33) agreed with Dutrochet that the propagation of stimuli in sensitive plants is effected by the water movements in the elements of the xylem. This same view was later emphasized by Sachs, Pfeffer and others.

The structural details of pulvini were probably first described and figured by Sachs (40, p. 793).

Brücke (7) studied the changes in the turgor relation of the pulvini during stimulation, and recognized that the curvature of the pulvinus of *Mimosa pudica* was connected with the flacidity of the responsive half of the pulvinus produced by the escape of water.

Millardet (34) and Bert (2) concluded that the changes of expansion in the antagonistic halves of the pulvinus were alike in both halves, but differed quantitatively, and also in progress of time.

Bert studied the rate of propagation of stimuli in sensitive plants.

Pfeffer has contributed a great deal to our knowledge of irritability. His "Die periodischen Bewegungen der Pflanzen" is one of the most important of his works (37).

Darwin (11) made very extensive studies on the sensitivity of plants. He was the first to apply the term "Nyctitropism" to the sleep movements in plants. The irritable movements of the cotyledons of various sensitives were emphasized by Darwin.

Gardiner (17) believed that in all irritable organs the movements are brought about in consequence of a definite contraction of the protoplasm of the irritable cells and that during such contraction some of the cell sap escapes to the exterior.

Haberlandt (18) put forth the view that the stimuli are carried by special cells in the phloem of the vascular bundles. Hanstein before Haberlandt came to the conclusion that the sieve tubes are structures similar to nerves. Haberlandt considered the carriers of stimuli to be special cells that are found only in sensitive plants. He described these cells as being 6 to 4.2 mm. long and of an average width of .18 mm. The walls of these cells are thin and are dotted; the dividing end walls are finely porous and penetrated by plasma threads. The cell contents consist of a thin plasmatic peripheral layer with a very large, round, or elongated nucleus. The contents of each cell contain mucilage, glucoside and resin. These cells, according to Haberlandt, are interwoven with the vascular bundles of stem, leaf, petiole and pulvini. The whole conducting system acts as one in which intercommunication exists, and one that is fused and through which hydrostatic waves pass and propagate stimuli.

Borzi (4) emphasized certain structural details of various sensitives—species of *Mimosa*, *Aeschynomene*, *Neptunia*—as aiding in the explanation of sensitive relations. He described (1) the structure of the peripheral regions—epidermis, hairs, etc.—as the parts that perceive stimuli directly; (2) The deeper conducting regions; (3) The pulvini as the irrito-contractile centers.

Mac Dougal (27), in his studies on *Mimosa pudica*, showed that Haberlandt's explanation of transmission of stimuli by the special cells of the phloem was not tenable. Stems from which the phloem region was removed were still able to transmit stimuli. "Excluding the hydrostatic theory of Haberlandt, at present it seems necessary to assume transmission by the tissues of the entire cross section."

Mac Dougal (28, 29) also studied the propagation of stimuli in *Biophytum sensitivum*. In this work he came to the conclusion that the path of the transmission of the stimuli is the parenchyma of the fibrovascular bundles and that the impulse is conducted plasmatically. He says "it seems quite possible that the protoplasm action plays a part in carrying the impulse from the point of reception to the motor organ, and that while hydrostatic disturbance does not constitute an impulse it may play a minor part in the transmission."

Nemec (36) concluded that stimuli are propagated by the plasma membranes of the cells, and by the intercellular connecting fibrils which come in contact with the plasma membranes.

Macfarlane (30) observed the flush changes in the tertiary pulvini of various sensitives, such as *Mimosa pudica*, *M. lupulina*, *M. sensitiva*, *Schrankia angustata*. "After stimulation of a leaflet and toward the close of the latent period a sudden flush travels centrifugally across the surface of the pulvinus. Immediately thereafter the leaflet contracts and the pulvinus previously of a whitish hue assumes a dull greenish aspect. This color change is due to migration of liquid into the upper region of the pulvinus."

Cunningham (10) fully described the pulvini of various sensitives—mainly *Mimosa pudica*—as motor organs. He states "The idea is erroneous, that in the opposing masses of tissue in pulvini, we have to deal with differences depending solely on invisible molecular structure, and not on the presence of any visible difference in organization.

Haberlandt (19) considered that the epidermal hairs found on sensitive plants especially those on the pulvini of *Mimosa* and the leaflets of *Biophytum* are receptors of stimuli. Experiments are given in support of this view. Shortly before Haberlandt's paper, Renner (39) had emphasized the presence of various kinds of hairs on sensitives.

The different works of Bose (5) that have appeared during recent years have added much to our knowledge of Plant Irritability. By means of very ingenious devices he has recorded accurately the various movements of some of the sensitive plants, especially *Mimosa pudica* and *Desmodium gyrans.*

Recently Linsbauer (25) made observations on propagation of stimuli in *Mimosa pudica.* He writes that propagation of wound stimuli (traumatic) can take place for considerable distances in the stem, even if all of the tissue outside of the wood is removed. He denies the presence of special conducting elements in the phloem.

The above brief historical review includes only some of the more important references on irritability. Other references will be quoted in the body of this work.

Distribution and Relative Sensitivity

Leguminosae

This family is very widely distributed in both hemispheres, from the cold, almost frigid, regions to the tropics, where a large number of genera grow in great profusion.

In the colder temperate regions, only a few members are found that are sensitive to any degree, and such sensitiveness is shown only in response to light stimulation, that is to nyctitropic stimuli and to the effects of rather intense sunlight. There is no response, or a very slight response, to other kinds of stimuli, such as mechanical, chemical, thermic, electric and other forms. Species of *Lathyrus*, *Vicia* and *Amorpha* extend northward to the Arctic Circle. The northern limit of some species of *Trifolium*, *Lotus*, *Astragalus*, *Phaseolus*, *Strophostyles* and *Petalostemum*, and also additional members of the three genera previously mentioned, is at a somewhat lower latitude. The leaves of these forms show the day and night novements, some of them, like *Trifolium*, to a very marked degree. Darwin (p. 349) examined eleven species of *Trifolium* and found that nearly every one showed nyctitropic movement in its leaves and also in the cotyledons in some of them.

The paraheliotropic response is well illustrated in some of these more northern representatives of the family. In *Strophostyles helvola*, the trailing wild bean, of eastern North America from Quebec southward, the leaflets of each of the tri-foliate leaves turn their edges toward the sun when the light is intense and the temperature approaches 32° C.

In the warm temperate regions an increasing number of Leguminosae show sensitivity. In a climate such as ours, with its warm, or almost sub-tropical summers, with temperatures not unusually during the day of 32 to 40° C., or even higher, and generally with quite a sudden drop of temperature after sunset, a marked effect on the vegetation is shown.

When there is a drop in temperature, it means that some plants must provide against the too rapid radiation of heat. This provision the leguminous plants show in their capacity to close the leaflets at night. Practically all the plants of this family in our region show this phenomenon. Of those which are native, or introduced here, are species of *Cassia*, *Gleditschia*, *Gymnocladus*, *Lupinus*, *Phaseolus*, *Medicago*, *Melilotus*, *Amorpha*, *Tephrosia*, *Robinia*, *Astragalus*, *Aeschynomene*, *Desmodium*, *Clitoria*, *Apios*, *Strophostyles*, *Pueraria*, *Amphicarpaea*, etc. All of these are sensitive to nyctitropism, and nearly all to paraheliotropism in that the leaflets turn their edges toward the sun when the light intensity gets above a certain optimum which varies with the different plants. Many of these plants also are sensitive to mechanical and other forms of stimuli.

Nyctitropic response is very well seen in the genus *Cassia* (Plate LVIII, Fig. 1-2), that is tropical in its distribution and is represented here by three outlying species. Night sleep of leaves is beautifully shown by the arborescent members mentioned above,—*Gleditschia*, *Gymnocladus* and *Robinia*. The last of these, the black locust, shows the phenomenon to good advantage. Shortly before sunset the leaflets, which during the day, if the temperature does not exceed 32° C., are in a horizontal position, begin to sink until in an hour or more they are back to back, having described an angle of 90°. The day position of leaflets, when the temperature rises to 32°–35°, or higher, is such that the edges are turned toward the sun, so that the incident rays of light fall parallel to the surface of the leaflets. The paraheliotropic movement therefore is above the plane of the horizon instead of below as seen in the nyctitropic response. The sensitive movements of the leaves of *Melilotus alba* are described by Wilson and Greenman (47), and those of other genera by Macfarlane (31).

In the sub-tropical regions the members of this family are abundant, with many species of *Cassia*, *Neptunia*, *Aeschynomene*, *Desmodium*, *Melilotus*, *Acacia*, *Calliandra*, *Prosopis*, *Schrankia*, *Mimosa*, *Desmanthus*, *Caesalpinia*, *Crotalaria*, *Indigofera*, *Sesbania*, *Arachis*, *Phaseolus*, etc. Practically all of these plants, as far as studied, are sensitive. In some, the sensitivity has been developed to a high degree. The genera that include some of the most sensitive plants of this family are here represented. A number of species of *Mimosa*, of *Schrankia*, of *Prosopis* and of *Calliandra* appear for the first time. In the tropics numerous species of these genera are found. In the sub-tropics many of the genera common in the temperature zones are also represented here. Some of these extend over 80 degrees of latitude and are found in both hemispheres, as for example, species of *Desmodium* which extend from south central Canada on the north to sub-tropical Australia and New Zealand on the south.

In the tropics the *Leguminosae* reach their climax of development, comprising a large number of genera and species. In the western hemisphere, Brazil is the center of distribution of the family. From this region there have been obtained species of *Mimosa*, *Schrankia*, *Cassia*, *Inga*, *Pithecolobium*, *Calliandra*, *Bauhinia*, *Sweetia*, *Zollernia*, *Exostyles*, *Caesalpinia*, *Peltogyne*, *Hymenaea*, *Parkia*, *Prosopis*, *Neptunia*, *Aeschynomene*, *Abrus*, *Vigna*, *Dolichos* and a number of smaller genera. Some genera include a large number of species,—e.g., *Mimosa*, more than 300 species; *Cassia*, over 300 species; *Calliandra*, nearly 100 species.

The abundance of leguminous plants in the tropical vegetation, and the very pronounced types of sensitivity exhibited by these, have been noted by many botanists and travelers to the Amazon Valley and other parts of the tropics. De Mello (15, p. 253) writes:—"The order of Leguminosae is here (Campinas, Brazil) a most extensive one, represented by very many genera and innumerable species, from small herbs to the tallest trees."

Richard Spruce (44) in "Notes of a Botanist on the Amazon and Andes" refers repeatedly to the abundance and great variety of different sensitive plants. He says (p. 257): "But of all orders by far the most abundant constituent of the flora of the Amazon is Leguminosae." (p. 258) "Sensitive plants, here called Sleepy Plants (Dormideiras), are here so common that almost every day I scratch my fingers or my shins against some thorny member of the group."

In reference to *Mimosa*, Wallace (46, p. 262) says: "Among the more humble forms of vegetation that attract the traveler's notice none are more interesting than the sensitive species of *Mimosa*. Where a large surface of ground is covered with these plants the effect of walking over it is most peculiar. At each step the plants for some distance round suddenly droop, as if struck by paralysis, and a broad track of prostrate herbage, several feet wide, is distinctly marked out by the different colour of the closed leaflets. The true sensitive plants are all low-growing herbs or shrubs with delicate foliage, which might possibly be liable to destruction by herbivorous animals, a fate which they may perhaps escape by their singular power of suddenly collapsing before the jaws opened to devour them."

In the Old World tropics many Leguminosae are found, but not so abundant as in the New World. Some genera, like *Mimosa*, *Cassia*, *Desmodium*, *Acacia*, *Prosopis*, *Parkia*, *Cynometra*, *Copaiba*, *Bauhinia*, *Sophora*, *Crotalaria*, *Indigofera*, *Astragalus*, *Aeschynomene*, *Vigna* and *Dolichos* have representatives in the tropics and sub-tropics of both hemispheres. Comparatively few genera are restricted to the Old World.

From the general survey on the distribution of the Leguminosae it will be noted that a few members extend to the Arctic regions, e.g., *Lathyrus maritimus*; several species of *Desmodium* reach their southernmost limit in Patagonia. More representatives of the family are found in the temperate zones, with a gradual increase in the number of genera and species toward the tropics where the leguminous plants flourish in great abundance.

The increase of temperature is correlated with an increase of the number of plants of this family and with the gradual advance in the sensitivity of the different members that show this phenomenon. Nearly the entire family shows night sleep of leaves. A considerable number move paraheliotropically. Other sensitive responses have been evolved gradually. A considerable number of leguminous plants of the temperate regions respond to mechanical, electrical, and chemical stimuli, but the movement is sluggish and not propagated for any great distance from the center of the stimulation. The plants that show the best response to the three methods of stimulation given are found in the tropics. The forms with the most highly specialized sensitiveness to the greatest number of stimuli are natives of the tropics, especially in the New World.

Mimosa pudica, the best known of the sensitive plants, is native to the Amazon Valley, but has been introduced into Asia and Africa, so that sensitive plants now abound in many parts of the eastern and western tropics. *Mimosa Spegazzini*, which is more sensitive than *M. pudica*, is also indigenous to the South American tropics. These two forms represent the acme of leaf sensitivity among the forms that are in cultivation and that have been studied. There may be others in the tropics that are equally sensitive, or even more sensitive, but which have not been studied.

Why the climax in the number of species of sensitives and in the high degree of irrito-contractile movements in the tropics? This can probably be attributed to the climatic conditions peculiar to the tropics. During the day, the temperature averages approximately 35° C. The atmosphere is rather humid with rainfall every afternoon during the wet season.

Bates (1) gives a very interesting account of the equatorial climate. He refers to the shortness of the twilight and the consequent rapid transition from day to night and from night to day. The deposition of dew on the foliage at night is very heavy. The nights are cool. In describing the daily rains he writes: "The heat increased hourly, and toward two o'clock reached 93° F. The leaves which were so moist and fresh in early morning, now became lax and drooping. On most days in June and July a heavy shower would fall some time in the afternoon. The cool sea breezes died away. The heat and the electric tension of the atmosphere would then become almost insupportable. The whole eastern horizon would become almost suddenly black. Then the rush of a mighty wind is heard through the forest, swaying the tree tops; a vivid flash of lightning bursts forth, then a crash of thunder, and down streams the deluging rain. After the rain heaps of flower petals and fallen leaves are seen under the trees."

The high temperatures by day, the sudden drop of temperature after sunset, the rapid transition from day to night, the heavy dew, the heavy daily rains with the accompanying winds, and dark skies (and the presence of browsing animals, as suggested by Wallace), all these factors aid in explaining the advantage resulting from closure of the leaves of the sensitives, and these further explain why so many of the Leguminosae have acquired this phenomenon of leaf movement.

Many genera probably once sensitive, now, owing to colder conditions as the plants were farther and farther removed from the tropics, have non-sensitive, or slightly sensitive species. Other genera that are distributed through colder regions and that are sensitive to day and night changes have not acquired sensitivity to ordinary stimuli, or may have lost sensitivity.

Oxalidaceae

This family in its distribution and sensitive relations, as well as in structural details, shows many similarities to the Leguminosae. The family is much smaller than the one previously described, comprising seven genera with about 250 species. The Oxalids are found in both hemispheres, with the largest number in the Western Hemisphere, extending from Northern Canada to the southern extremity of South America. In the Old World the range is from northern Europe and Siberia to New Zealand in the Southern Hemisphere.

Oxalis Acetosella is the most northern representative of the genus and of the family, being found in northern and middle Europe, eastward through Siberia and south to the Himalaya Mountains. In the New World, this plant is distributed throughout north central Canada, in eastern Canada, south in the mountains to North Carolina. It has long been a favorite plant for study, since it shows very striking nyctitropic movements of its leaflets. It also shows slight paraheliotropic response, and response to other stimuli.

In our region, seven species of *Oxalis* are found; two of these—*O. corniculata* and *O. stricta*—are very common. The leaflets of the different species move nyctitropically and paraheliotropically, but exhibit very sluggish movements when stimulated by mechanical, chemical, or other means of stimulation.

In the sub-tropics there is a marked increase in the number of species of *Oxalis*, and other genera—*Biophytum* and *Averrhoa*—are also represented. *Biophytum dendroides* is found from central Mexico southward. *Averrhoa* is distinctly a tropical genus, but both of the species extend into some of the sub-tropical regions of Asia.

By far the largest number of species of Oxalidaceae is found in the tropics of both hemispheres, with the majority in the New World. In South America the genus *Oxalis* is represented by more than 200 species, including the most sensitive members of this genus. Of those in cultivation such sensitive forms as *O. bupleurifolia*, *O. scandens* and *O. Ortgiesii* are native to the New World tropics. Comparatively few species of *Oxalis* are found in the Eastern Hemisphere. The genus *Biophytum* is essentially tropical in distribution in America, Asia, and Africa. *B. sensitivum*, the most sensitive species of this genus that has been studied, is found in both the Old and the New World. *B. dendroides*, scarcely less sensitive than the above species, is indigenous to the American Tropics. *Averrhoa*, a small genus, but including several species, e.g., *A. carambola* and *A. bilimbi*, that have been studied quite extensively, is found wild or cultivated in the tropics of both hemispheres.

Practically all the tropical species that have been studied exhibit irrito-contractility in their leaves. Not only are they sensitive to day and night conditions, but when stimulated mechanically, chemically, electrically or otherwise the leaflets close, dropping back to back, below the plane of the horizon. The sensitivity varies for the different species, and, as compared with the most sensitive members of the Leguminosae, is less marked.

In the Oxalidaceae, as in the Leguminosae, the range of distribution extends from the Arctic Circle on the north to the colder temperate regions on the south, with by far the largest number of representatives in the tropics.

The relative sensitivity shows a gradual increase from the species found in the colder regions to those types that exhibit the highest degree of sensitiveness and which are distributed throughout the tropics.

The probable reasons why the tropical forms of this family have acquired sensitivity that is in advance of those in the cooler regions are the same as those brought out for the Leguminosae. The two families suggest many similarities. As indicated, the range of distribution is practically identical with the climax in the number of species in the tropics for both groups. In both we find compound leaves (a few exceptions in the Leguminosae), that are provided with primary pulvini at the bases of the petioles, a secondary pulvinus at the base of each leaflet, and if the leaf is bi-pinnately compound, as in *Mimosa pudica*, a tertiary pulvinus at the base of each secondary leaflet. Both families show similar response to stimuli.

In the Leguminosae and in the Oxalidaceae, the phases of stimulation are alike. In both: (1) there must be an optimum environment in order that response may be shown. Such environal factors as suitable temperature, sufficient light, a certain amount of oxygen in the surrounding atmosphere, and also the health and vigor of the plant used in the experiment, all have a very important bearing on the response to be obtained. (2) given that the environment of the plant is favorable, if a specific stimulus, which acts as so much energy, is applied to irrito-contractile parts of the plant, a response follows. (3) The response of the irrito-contractile parts is not immediate, but a time interval, the latent period, elapses before there is a change of position of any of the organs of the plant. During this period, the stimulus is presented to and perceived by the plant parts that are irritable. Owing to varying degrees of irritability in different organs of the same plant, or of different plants, the duration of the latent period varies within quite wide limits—from 1/4 second, or even less under optimum conditions in *Mimosa Spegazzini*, to an hour or more in the feebly sensitive members of the two families under discussion. (4) Following a certain time interval after the stimulus has been applied response in the irrito-contractile organs follows. This may be slow and steady or rapid and extensive in the plant. There can only be response if the stimulus, which has been applied to the plant, was of a certain definite intensity, and if it acted for a sufficiently long period of time. A weak stimulus, presented for a short time, may stimulate to a certain degree, but there may be no response in the irrito-contractile organs. In such case there has been an insufficient intensity of excitation to bring about response. Response follows on applying the weak stimulus for a longer time, or applying it a number of times to the irritable plant parts.

(5) Hence here a summation of stimuli is necessary to produce a response. In many species of Leguminosae and of Oxalidaceae that are very slightly sensitive a considerable number of stimuli are required to bring about closure of leaflets. (6) The stimulus is propagated from the center of stimulation to other parts of the plant; the distance through which it is carried being determined by the intensity of the stimulus, the time during which it acts, and the relative irritability of the plant used. (7) After contraction of the irrito-contractile parts has ceased, there follows the neutral period, during which there is no evident movement of the parts of the plant involved in the stimulation act. (8) After the neutral period the contracted parts re-expand and assume the original position; this phase is called the re-expansion period.

The eight phases just given comprise a complete stimulation act, and are identical for the Leguminosae and the Oxalidaceae.

In illustration of the above eight phases, the following illustrative example might be cited in a stimulation act for *Mimosa pudica*:—When a mechanical stimulus in the form of a forceps pinch is applied to, say, the tenth secondary leaflet from the apex on a left-handed primary leaflet, an extremely short latent period ensues, followed by rise of the stimulated leaflet and almost instantly thereafter by rise of the neighboring leaflet. Thus irrito-contraction of the pair initiates movement in the leaf. A propagation of the stimulus then travels in two directions; one more rapidly in an upward direction causing successive closure of all of the leaflets to the tip; another more slowly in downward direction causing similar closure. Such propagation is effected under optimum summer conditions in eight seconds to the terminal pair of secondary leaflets, and ten seconds to basal pair. The distances through which the stimulus is propagated are equal. This, therefore, indicates that the rate of propagation of stimuli is slightly higher in a centrifugal than in a centripetal direction. When the stimulus reaches the base of this primary leaflet a flush change passes over the pulvinus, followed by a slight converging of the secondary petioles. Burnett and Mayo (8, p. 81), Paul Bert (2, p. 12) and successive observers noted the flush changes of the secondary and primary pulvini. The tertiary pulvini also show these changes just before the pairs of secondary leaflets close. Propagation of the stimulus is rapidly effected down the petiole to the primary pulvinus, and a drop of the entire leaf follows in two to three seconds after the change in the position of the secondary petioles had taken place.

The stimulus next travels into the leaflet adjoining and almost at the same time into the leaflet opposite. A short time thereafter the stimulus reaches the remaining leaflet of the four. In all three leaflets the secondary leaflets close, the stimulus moving toward the apex of each leaflet. Thus in twenty-five to thirty seconds after the stimulus had been applied all the secondary leaflets are closed.

The neutral period which now ensues may last on an average of three to five minutes. The leaflets then re-expand and the whole leaf rises to its original position. The re-expansion period is ten or eleven minutes. Thus are illustrated successively a stimulation act, latent period, response of leaflets, propagation of stimuli to successively removed centers, neutral period and re-expansion period.

Having noted the similarity in sensitive movement and in distribution in the Leguminosae and the Oxalidaceae, the histological study of the leaves of certain members of these families will be taken up in the succeeding chapters. The plants studied include forms that are feebly sensitive, others that are sensitive to a marked degree, and others that are very highly sensitive.

A very suggestive feature of histological interest in the Leguminosae and the Oxalidaceae is the presence of crystals. These are found in practically all members of these families. In the individual plant they are present in leaves and stems.

The writer made a careful study of the crystal distribution in the different sensitives, from forms that are scarcely sensitive to those that exhibit the most specialized types of sensitivity.

Plant crystals have long been known and studied. Malpighi (32) was the first to observe "crystal clusters" in plants.

Von Leeuwenhock (23) noted several forms of crystals and described these briefly.

Scheele (41) was the first to make a chemical investigation of plant crystals, and indicated that they are composed, for the most part, of calcium oxalate.

These earlier investigations of crystals were made on plants not included in the families here studied.

Meyen (33) was probably the first one to study rather carefully the crystals found in the cortex of the stem of *Robinia pseudacacia.* The crystals were studied from the chemical standpoint. He agreed with Scheele that the crystals are composed of calcium oxalate. Meyen observed that the crystals are always contained in cells, and not in intercellular spaces, as the preceding workers believed. In this work no reference is made to the distribution of crystals, but their forms and shapes are described.

Holzner (21) takes up a general study of crystals, with brief reference to those of *Robinia pseudacacia.* This work is rather important in that it includes a summary of all the literature on crystals up to 1864.

Poulsen (38) briefly describes the crystals found in various papilionaceous plants, such as *Apios*, *Phaseolus* and *Dolichos.* In these, according to Poulsen, crystals are especially abundant in the characteristically swollen bases of the petioles (pulvini) and in the thickened flower stalks. They are not found in the epidermis but in the parenchyma tissue beneath, in the parenchyma cells of the fibro-vascular bundles and in the central pith-like tissues. Those in *Phaseolus*, he says, are large, single crystals which have no definite relation to the axis of the organs. They are elongated, prismatic, rhombic in cross section, and are invested by a cellulose mantle which is usually quite thick. When the crystals are too small to touch the cell walls, the cellulose envelope at the ends of the crystals is prolonged into cellulose beams which are in contact with the cell membranes.

Coester (9), in his study of Mimosas, states that calcium oxalate crystals are deposited in all plant parts of these sensitives. Two forms of crystals occur—as individual twin crystals and as crystal clusters. The former kind is found in all the forms examined, occurring as monoclinic plates, or styloid-like crystals. Coester further says that the presence of the crystals is generally associated with the vascular bundles. Here they are enclosed in the parenchyma cells around the bundles. The formation of crystals is so abundant that over the larger veins of the leaves every cell contains a crystal. When viewed from the surface, the vascular bundle appears to be covered by crystals, arranged in regular series. The crystal system is usually found in connection with even the smallest bundles. Crystals are found in the stems as well as in the leaves.

Solereder (43, p. 266) refers to the wide distribution of crystals in the Leguminosae, and says two types are present—crystal clusters, which are very rare, and solitary crystals, which have either the ordinary rhombohedral shape, or that of small rods or styloids. "The ordinary rhombohedra occur especially in the tissue accompanying the vascular bundles of the veins, in the chambered fibers of the bast and wood, and also in the primary cortex. Those crystals which are rod-shaped, or resemble styloids are especially peculiar to the mesophyll, but occasionally occur in the epidermis of the leaf, in the crystal-containing chambered fibers of the bast and also accompanying the sclerenchyma of the veins. They are, strictly speaking, not solitary, but hemitropic crystals, consisting of two or more individual crystals, arranged with their longitudinal axes in approximately the same direction."

Solereder (43, p. 171) also refers to the crystals of the Oxalidaceae, as occurring in the form of raphides, clustered crystals and solitary crystals, but says nothing of the distribution of the crystals in the different members of the family.

LEGUMINOSAE

Pueraria Thunbergiana

This plant is native to the tropical and sub-tropical regions of Asia, but grows very well in the temperate regions, where it has been introduced as a hardy, perennial twiner. It bears large trifoliate leaves that show paraheliotropic and nyctitropic responses to a marked degree. When the temperature rises to 32° C. or higher, the leaflets turn their edges toward the sun. On account of the huge size of the leaflets, often 7 or 8 inches long, and 4 or 5 inches wide, the paraheliotropic effect is very striking. The night sleep of the leaflets is equally prominent. The leaflets drop in the nyctitropic movement.

Leaflets. The crystals in the leaflets are of the styloid type (Plate LIX Fig. 3) and are mainly distributed along the veins, with a few scattered through the mesophyll tissue. The crystals along the veins are arranged in broken lines, with rather wide gaps between the crystals. Each crystal is rod-shaped with short projections at each end, at right angles to the long axis of the crystal. The average dimensions of the crystals are 11.4 microns x 5.65 microns.

Pulvini. Crystals are found in the primary pulvinus and in the secondary pulvini of each leaf. The type of crystal is similar to that found in the leaf (Plate LIX, Fig. 4). The greater number of crystals in the pulvini are present just around the vascular bundle cylinder, with scattered crystals throughout the cortex from the bundles to the epidermis. Here and there a crystal is found with a faint line across its middle portion, at right angles to the long axis. This is the beginning of the twin-crystal type so characteristic of the more sensitive plants.

Petiole. The rod-shaped, or styloid-like crystals are also present in the petioles, but in smaller numbers relatively than in the pulvini. In addition to these crystals there occur in the petiole a second type, a rhombohedral form. The latter kind of crystal occurs along the bundles in the endodermal region. Here the crystals are arranged in discontinuous lines. The individual crystals are considerably smaller than the cells in which they occur, each crystal occupying about one-half of the lumen of the cell in which it is contained. The longer axis of these crystals is parallel to the axis of the vascular bundles.

Bauhinia diphylla

The genus *Bauhinia* belongs to the sub-family Caesalpinioideae of the Leguminosae, comprising about 150 species, distributed throughout the tropics and the sub-tropics of both hemispheres. *B. diphylla* is a native of India, growing at rather high elevations. This plant, which is often grown in green houses, bears simple, deeply two-lobed leaves. The single leaf probably represents the terminal leaflet of an originally compound leaf. At the base of the petiole there is a primary pulvinus, and at the base of the lamina a secondary pulvinus, both of these only slightly thicker than the petiole. The leaves are very feebly responsive to mechanical stimuli. Only after numerous stimuli (as many as 15 or 20) do the two lobes of each leaf rise through a small angle. Marked nyctitropic response is shown, the lobes at night moving upward through an angle of 80 to 90 degrees. There is also a slight drop of the entire leaf from the day position.

Leaf. Scattered throughout the mesophyll of the leaves are a number of conglomerate crystals (Plate LIX, Fig. 5). In addition to this type there are prismatic crystals arranged in discontinuous single rows along the veins. This latter type shows considerable variation in the shape of the individual crystals. Some are irregularly rounded and in general outline resemble the scattered conglomerates; others are regular prisms, usually four or six angled. The hexagonal form is rather rare. The distinct cross-line, so well seen in crystals of the more sensitive plants, is indicated very faintly on a few of the six-sided crystals. The crystals are small, rarely more than 9 microns long. The breaks in the lines of crystals are shown (Plate LIX, Fig. 5).

Pulvini. In both the secondary and the primary pulvini conglomerate crystals are scattered through the cortex, but none along the vascular bundles.

Petiole. In the petiole lines of crystals occur regularly in the endodermal cells surrounding the vascular bundles. In some cases only a few crystals occur, or there may be twenty or even more crystals in a line, then a break, then another line and so on. The crystals are rather small as compared with the size of the cells in which they are found. A thin protoplasmic sheath surrounds each crystal.

Gleditschia triacanthos

The genus *Gleditschia* is essentially tropical and sub-tropical in its geographical distribution, being represented in tropical Africa, tropical and sub-tropical Asia, sub-tropical Argentina, and in eastern and southern North America.

G. triacanthos, the Honey Locust is indigenous over a wide region from southern Canada to Texas. The pinnately compound leaves show slight nyctitropic movement. In no case has the writer observed a drop of the leaflets of more than 30 degrees. Rather feeble paraheliotropic movements have been observed, and no response to other forms of stimulation. It is quite probable that this outlying species of a genus, that is tropical and sub-tropical in its distribution, has lost its sensitivity. The protection afforded by a closure of the leaflets is not necessary in this plant. The small leaflets are sufficiently protected against a too rapid radiation of heat by the well developed cuticle.

Leaflets. Broken lines of crystals abound along the veins of the leaflet. One to six or eight lines of crystals are present, according to the size of the vein. In shape the crystals are quite regular, mostly four-angled, and relatively few six-angled. The size of the crystals is extremely various, from the smallest ones less than 3 microns long, to the largest ones, 15 microns long. The crystals show no definite polarity of arrangement with respect to the direction of the vascular bundles. In many cells the longer axis of the crystal is at right angles to the greatest length of the cell containing the crystal.

Pulvini. In the secondary pulvini, as well as in the primary pulvinus of each leaf, are found the same types of crystals as in the leaflets, but these are more scattered in their arrangement. There are few conglomerate crystals present in the cortex of the pulvini.

Petiole. The crystals of the leaflets, the pulvini, the midrib and the petiole of the honey locust are very similar throughout in shape and arrangement, but with a greater number of crystals in the leaflets and in the petiole than in the pulvini. The crystals are imbedded in a proto-plasmic sac or envelope which is not as dense as the envelopes in the more sensitive plants are, as is indicated by the rather light stain of the enveloping sheaths when protoplasmic stains are applied.

Gymnocladus dioica

Gymnocladus is a small genus, including two species, *G. chinensis* of eastern Asia, and *G. dioica* (the Kentucky Coffee Tree) of eastern North America, from southern Canada to Oklahoma. The bi-pinnately compound leaves of the latter species show night sleep very strikingly. When observed at night the leaflets are in a position that is almost vertical, having moved downward. The movement and the position of the leaflets at night are very similar to those of *Robinia*. The distribution of the two plants is almost exactly identical with a slightly wider range for the Kentucky Coffee Tree. The paraheliotropic response is not as marked as it is for the leaflets of *Robinia*. In the latter the upward movement begins when the temperature reaches 24° to 27° C. and when there is bright sunlight, but *Gymnocladus* requires a higher temperature to initiate the movement. The maximum angle through which the secondary leaflets of this tree move is less than that of the black locust. The writer was unable to get any visible response in the leaflets to mechanical stimuli, but noted a slight drop of the secondary leaflets when 50% sulphuric acid was applied to the tertiary pulvini. The primary pulvinus of the leaf is not well defined, appearing as a slightly swollen, clasping leaf base, and shows no irrito-contractility when stimuli are applied to it.

Leaf. The crystals in the secondary leaflets are found along the veins and are arranged in discontinuous lines. A single line is present along the smaller veins. With an increase in the size of the veins there are introduced additional ranks of crystals until in the midrib there are present 7 to 9 broken rows of crystals. The crystals vary considerably in shape and size, with the four angled prismatic type in greatest abundance, and among these relatively few hexagonal prisms. The latter in some cases indicate a very faint line across the middle of each crystal. The hexagonal forms are rather small, with an average length of 12.3 microns and a width of 6.83 microns. Conglomerate crystals are present in the bundle of the midrib, but none in the mesophyll of the leaflets.

Pulvini. Two kinds of crystals are present in the tertiary pulvini—the quadrangular type noted in the leaflet bundles, and an abundance of conglomerates The regular prismatic forms are found in the endodermal region, the lines being continuous from the midrib bundles. The conglomerate crystals are found in the cortex of the pulvini, where they occur in greater number on the dorsal side of the pulvini, and are also present in the vascular bundles.

In secondary pulvini the crystal relation is similar to that described for the tertiary pulvini (Plate LX, Fig. 6), and the same is true for the primary pulvinus, but here very few crystals are present. Of the regular prismatic type, none indicated the hexagonal form.

Petiole. In the secondary petioles we again note the regular prismatic crystals arranged in fairly continuous lines in the endodermis. As in the leaflets, the shape of the crystals varies, with here and there a hexagonal crystal present. Conglomerate crystals are also present in the inner cortex and in the vascular bundles. These, like the prisms, show variation in shape, with transitions toward the prismatic forms. The crystal distribution in the primary petiole of each leaf is similar to that in the secondary petioles, with fewer crystals relatively and more breaks in the lines of crystals than in the secondary petioles.

Robinia Pseudacacia

The black locust is native to the eastern United States, from Pennsylvania southward to Georgia, westward to Oklahoma, but has been naturalized as far north as southern Canada. The imparipinnate leaves of this tree show pronounced sensitivity to nyctitropic and paraheliotropic stimuli. At night the leaflets drop so as to be placed in an almost vertical position. The movement under the effects of intense sunlight is upward. In midsummer in bright sunlight when the temperature reaches 27° C. or higher, the leaflets of the black locust are directed toward the light at an angle to the vertical of 35 to 40 degrees. Later in the day with a decrease in intensity of the sunlight and a gradual drop in temperature the leaflets sink, until by 5 o'clock, as observed in a number of cases, the leaflets were nearly or quite flat. Then there followed a gradual sinking of the leaflets below the plane of the horizon, by 7:30 or 8 o'clock they will have attained the night position, making a very slight angle to the vertical. To mechanical or chemical stimuli, *Robinia* is feebly responsive. If when the leaflets are expanded shock stimuli be applied to them, a slight drop will follow after a number of stimuli have been given. Here then a summation of stimuli is necessary to bring about response.

Leaflets. Along the larger bundles of the leaflets two or three quite regular rows of crystals are found. Along the small veins there is a single line of crystals that is broken here and there. The crystals are mainly of two kinds; one that is small, quadrangular in surface view, seldom more than 7.6 microns long and 6.5 microns wide. These crystals lie near the middle of cells of considerable size, each crystal filling less than one third of the cavity of the cell in which it is contained. The other type of crystal is hexagonal is outline, 15.2 microns long and 7.6 microns wide. Occasionally a crystal is noted in the chains along the bundles that shows a line across its middle. The two types of crystals are found in the same line with rather more of the hexagonal prisms. The individual crystals of both kinds show quite wide variations in shape and size. The crystals are imbedded in protoplasmic sheaths or envelopes.

Pulvini. The secondary pulvinus of each leaflet shows rather a wide cortical region in which crystals are distributed—relatively few toward the epidermis and a considerable number just around the bundle, in the endodermal region. The crystals are nearly all of the styloid or rod shaped type with pronounced transversely projecting processes at both ends. These processes project from both sides of the ends and seem to be of a different composition from the elongated portion of the crystal, for, usually they take a slight stain when protoplasmic stains are applied to the crystal containing cells, while the rest of the crystal remains unstained. Some of the crystals show the partition lines acrsos the middle, as was indicated for some of the larger six angled crystals in the leaflets. The styloid crystals, on an average are of about the same in length as the hexagonal type but quite narrow, from 1.8 ot 5 microns side. In addition to the elongated form few quadrangular crystals occur in the pulvini. In the endodermal region the crystals show a certain polarity of arrangement, in that the longer axes of most of the crystals are in the same direction as that of the bundles.

In the primary pulvinus of the leaf the crystal distribution differs from that of the secondary pulvini in the smaller number of crystals present in the former.

Petiole. Both the elongated and the quadrangular types of crystal are found in the petiole, with a larger number of the latter kind. Both are arranged in discontinuous lines in the endodermal zone. The styloid type is most abundant in proximity to the pulvini. (Plate LX, Fig. 7).

Desmodium rotundifolium

The large genus *Desmodium*, of more than 150 species, is widely distributed throughout the tropics of both hemispheres. Representatives of the genus are also found in the sub-tropical and temperate regions. In North America, Desmodiums are found from southern Canada southward. Twenty species are included in the range covered by Gray's Manual. All of these species, as far as studied, show sensitivity in their leaflets. The degree of sensitivity varies considerably with the different species.

D. rotundifolium, of the four species studied, both from the crystal relation and the relative sensitivity, can well be considered as the least specialized. This species is found in the eastern United States from the Canadian border, southward to Louisiana. It is usually found in rather dry woods, where it creeps along the ground. The prostrate habit is given by Macfarlane (30, p. 202) as a reason for the sluggish irrito-contractility of this species as compared with other species of the genus. "*Amphicarpaea*, *Desmodium canescens* and *Desmodium panicu-*

latum are all upright growers, and are therefore exposed in their leaflets to the full effects of night cold and heat radiation from the tissues, and I believe that this may largely explain why in evolutionary development they have become much superior to *Desmodium rotundifolium*, whose long, sucker-like shoots run along the ground, and give off leaflets that nestle among the surrounding herbage."

D. rotundifolium shows night sleep in its leaflets and also paraheliotropic movement. The leaflets are slightly sensitive to mechanical and thermal stimuli. Both of these forms of stimulation cause a drop of the leaflets through a relatively small angle but only after a long latent period.

Leaflets. Crystals are present in the endodermis of the orbicular leaflets. The crystals, which are arranged in lines with occasionally a small gap in the crystal cell continuity, are small prisms, mostly of the six-angled type. The variation in shape and size of crystals is most marked. The average size for a number of the crystals is 9.5 microns long and 5.7 microns wide; the average dimensions of the crystal cells are 19.5 microns long and 14.2 microns wide.

Pulvini. In both primary and secondary pulvini irregular crystals are scattered throughout the cortex. These vary in shape from forms that approach the conglomerate type to others that are quadrangular, and relatively few that are hexagonal, but more elongated and not as wide as the hexagonal forms in the leaflets.

Petiole. The crystals are of the same kind and show similar distribution to those in the leaflets.

Desmodium Dillenii

This species is very common in the eastern half of the United States. From the few experiments made, the writer considers this plant to be close in its sensitive action and relation to *D. rotundifolium*, but slightly more sensitive, in that when the leaflets are stimulated mechanically, they drop after a shorter latent period than in the species previously described. The angle through which the leaflets drop is also greater than in the former species.

Leaflets. The crystal distribution in the leaflets of this species resembles rather closely that of *Desmodium rotundifolium*, but the crystal lines along the veins show fewer gaps. The crystals are larger relatively to the size of the cells in which they are contained. The hexagonal prismatic form is more in evidence than in the former species, and in the pulvini are fewer crystals than in *D. rotundifolium.*

Desmodium paniculatum

In distribution, in relative sensitivity, and in histological details this species resembles very closely the two Desmodiums just described. In the crystal sheaths of the leaflet bundles there is a decided increase in the twin rhombohedral crystals. The line across the middle of these six angled crystals is more evident in a greater number of crystals than for any plant studied so far. The crystals on an average are considerably larger than in *D. Dillenii* and *D. rotundifolium.*

Desmodium canescens

Of the native species of *Desmodium* studied, this is the most sensitive in its leaf movements. Both the nyctitropic and the paraheliotropic responses are most marked. When mechanical stimuli are applied the leaflets drop after a rather long latent period. In one experiment the median leaflet was stimulated by giving it a slight blow. After a latent period of 80 seconds, it dropped through an angle of about 20 degrees; 90 seconds after the first stimulus a second was given, followed by a further drop of the leaflet. A third and a fourth stimulus were applied with a 90 second interval between them. In all as a result of the four stimuli the leaflet dropped about 70 degrees. Dr. Macfarlane (30) states that the leaflets respond to chemical stimuli. A 6% ether solution applied to 8 median leaflets caused contraction in all of them. A drop resulted equally whether the ether was applied to the secondary pulvini or to the general leaf surface. A latent period of 1 to 1½ minutes followed the application of the ether.

Leaflets. The crystals along the leaflet bundles are arranged in fairly continuous lines, with few breaks in the continuity of the crystal cells. The more uniform regularity in the shapes of the crystals and their larger size, as compared with those in the other species of *Desmodium* is very marked (Plate LX, Fig. 8). The majority of the crystals are of the six angled prismatic type, with the partition line across the middle of most of the crystals evident. Polarity of arrangement of the crystals is more marked than in the other species of *Desmodium.*

Pulvini. Elongated, prismatic crystals are scattered throughout the cortex in both primary and secondary pulvini. In the endodermal region of the pulvini a greater abundance of crystals is present than toward the surface, and here in addition to the elongated type, the kind so typical for the leaflets is noted.

Desmodium gyrans

Next to *Mimosa pudica* there is probably no sensitive plant that has been studied more extensively than *Desmodium gyrans*. The autonomous movements of the lateral leaflets have been known and studied for several centuries. These oscillating movements are often so rapid that they may be readily followed with the naked eye. The larger terminal leaflet performs well marked nyctitropic, as well as less noticeable oscillating movements. The irregular twitching movements of the lateral leaflets have given the name Telegraph Plant to this species.

Leaflets. The crystals in the bundles of the lateral and the terminal leaflets are very abundant, forming continuous lines. The number of lines varies with the size of the veins with which they are associated. The crystals are practically all of the very regular six-sided, prismatic type. There is a greater abundance of crystals in the lateral than in the terminal leaflets.

Pulvini. In both primary and secondary pulvini, the kinds of crystals and their distribution in the cortex are similar to that described for *D. canescens*. The elongated rhombohedral type of crystal is here the dominant one.

Petiole. A great abundance of regular prismatic crystals is present along the vascular bundles of the petioles. So great is the number of these crystals that they form a broad continuous sheath over the bundles. In nyctitropic response the petiole moves upward from the day position.

Amphicarpaea monoica

This common leguminous plant, the Hog Peanut, abounds in eastern North America from Canada to Mexico. The thin tri-foliate leaves are sensitive to light and to mechanical stimuli. The day and night positions of the leaflets are fully described by Schively (42, p. 308). Usually 4 to 5 mechanical stimuli are required to cause the leaflets to drop through an angle of 70 to 75 degrees. Leaflets that have been so stimulated will recover the original position in 13 to 16 minutes. The latent period, the summation required, the response period, the neutral period and the re-expansion period, are all about the same, or slightly shorter, as for *Desmodium canescens*.

The crystal distribution in *Amphicarpaea monoica* is emphasized by Schively (42, p. 358), who writes:—"In early development numerous crystals appear in the cells forming the inner row of the cortex of the stem. These ultimately constitute a distinct crystal sheath. The crystals are somewhat prismatic in form, possessing an apparent partition across the middle. These twin structures occur also round the vascular areas of the leaves, and also in the cortex of the pulvini."

The writer compared very carefully the crystals in the first formed, simple, opposite leaves, and the later formed pinnately trifoliate leaves, and found the crystal system better developed in the compound leaves. The crystals are more abundant, larger, and the crystal lines are more continuous than they are in the simple leaves.

From experiments made on the relative sensitivity of the two kind of leaves, the writer found the simple leaves more sluggish in their movements. These experiments were made May 14, when the simple leaves were fully expanded, while the compound leaves were not fully developed. The younger condition of the compound leaves may explain their apparent greater sensitivity, for younger leaves always show quicker response than do those that are older.

Cassia chamaecrista

This species of *Cassia* and two others are northern outlying representatives of a large tropical and sub-tropical genus, which includes more than 300 species. *C. chamaecrista*, the Partridge Pea, is found in sandy soil throughout the eastern United States from New England southward and westward to Texas. This form is quite abundant in New Jersey, where it is often found growing with the next species, *Cassia nictitans*, from which it can be distinguished by its larger flowers. The Cassias are remarkably sensitive in their leaves, representing the most highly developed types of sensitivity among our native sensitives. The leaves of *C. chamaecrista* are evenly pinnate with 9 to 15 pairs of leaflets on each leaf. A short almost sessile gland, 1 mm. high, is present on the petiole, close to the primary pulvinus. The top of the gland is elliptical, or oval, in outline, slightly concave, and about 1 mm. long. The primary pulvinus is well developed, as are the secondary pulvini at the bases of the leaflets. The gland, as well as the leaflets and the pulvini, are irritable. In the superficial cells of the gland the appearance is as in the pulvini, to be described later—there being rather dense masses in each cell (Plate LXV, Fig. 26).

The night sleep of the leaves of the Partridge Pea is very pronounced, the leaflets folding upward and the whole leaf drops through an angle of 65 to 75 degrees. The paraheliotropic response is equally marked. At a temperature of 21 to 23° C. the leaflets are fully expanded; when the temperature rises to 26 or 27° C. the leaflets close ½; at 31 to 32° C. the leaflets are ½ closed, and at 33 to 35° C. complete closure is observed, with a drop of the entire leaf.

Not only are the leaves of *C. chamaecrista* sensitive to light stimuli but also to other forms of stimulation. When leaflets are stimulated mechanically they begin to close after a latent period of 3 to 4 seconds, at a temperature of 27° C. after the first stimulus the leaflets close ⅓ and the leaf drops through an angle of about 25 degrees, the movement continuing during a period of 1 minute. A second stimulus is then applied and after a latent period as before the leaflets close ⅔ that is they will have moved through an angle of about 60 degrees; the leaf drops through an angle of 15 degrees. On the application of a third stimulus, a latent period of 2 to 3 seconds ensues, the leaflets move through an additional angle of 20 degrees and the leaf drops 8 to 10 degrees further. The leaflets are now almost closed and additional stimuli cause no change in their position. Here, as for *Desmodium canescens*, a summation of stimuli is required, but fewer stimuli are needed than in the latter plant to cause closure of the leaflets. The leaf re-expands in 14 to 18 minutes. The leaflets are equally sensitive to chemical stimuli.

That the gland on the petiole is an irritable center was shown in experiments with certain chemicals by Professor Macfarlane (Lecture Notes). When he placed a drop of turpentine or alcohol or 60 p.c. sulphuric acid on the basal gland of a leaf with 13 pairs of leaflets, the upper 8 pairs closed rapidly, the lower 5 passed slowly through an angle of 40 degrees.

Leaflets. Continuous lines of crystals are present along the veins of the leaflets—two to five rows according to the size of the vein. The crystals are large, regular, hexagonal rhombohedra, and are so placed in the cells as to have the longer axes of most of them parallel to the direction of the vascular bundle with which they are associated. The average size of the crystals is 15.8 microns long and 9.4 microns wide. Each crystal cell contains one crystal, and is one-third larger than the crystal itself. The protoplasmic sheath in which each crystal is imbedded stains deeply with protoplasmic stains, such as eosin or aniline blue.

Pulvini. There are relatively few crystals present in the pulvini. These are all of the regular type found in the leaflets, and are located in the endodermal region, immediately around the bundles.

Petiole. The crystals in the petiole and midrib are so abundant as to form a crystal sheath that covers the vascular bundles, the main crystal development being found on the ventral side of the petiole. The crystals are of the regular type noted in the leaflets. Branches of crystal cells extend into the bases of the petiolar glands and can be traced to a distance representing ½ the height of each gland. The crystal cells do not have the same regular continuity that they have in the crystal lines of the petiole, but are more broken.

Cassia nictitans

This species, the Wild Sensitive-Plant of the eastern United States, shows close similarities, in its structural and histological details and in its irritability, to *Cassia chamaecrista.* Each pinnately compound leaf bears 8 to 20 pairs of leaflets. Britton (6, p. 529) states the number to be 12-44. The larger number probably applies to plants growing further south. On the upper surface of the petiole, near the basal pair of leaflets, a slightly-stalked gland is borne. The gland in this species, as in the last, is an irritable center.

The movements of the leaves under nyctitropic and paraheliotropic stimulation are like those described for *Cassia chamaecrista*, except that this species is slightly more sensitive, not only to light stimulation, but also to mechanical and chemical stimuli.

The illustration (Plate LVIII, Fig. 1) shows the expanded leaves of young plants grown in the green houses. In night sleep (Plate LVIII, Fig. 2) the leaflets are folded upward, back to back, and the leaves have dropped from the day position.

The upward movement of the leaflets in paraheliotropic stimulation begins at a slightly lower temperature than for *C. chamaecrista.* Here the movement begins at 24° to 26° C. in bright sunlight. As the temperature rises, the movement continues until a maximum closure is reached at about 32° C.

Under optimum environal conditions two mechanical stimuli cause a maximum closure of the leaflets and a drop of the entire leaf. After one stimulus has been applied there follows a latent period of 3½ seconds, and then a closure of the leaflets through an angle of 40 degrees. During the upward and slightly forward movement of the leaflets, the midrib of the leaf stimulated drops 35° to 40°. If a second stimulus is then applied, the leaflets close further through an angle of 30° and the midrib drops 10 to 15 additional degrees. A third stimulus brings very slight additional movement. The leaf so closed re-expands in 9 to 12 minutes.

Macfarlane (30, p. 201) proved that when carbonate of ammonia is applied to the petiolar gland, closure of the leaflets on that same leaf follows.

Leaflets. The regular lines of crystal cells along the veins stand out very clearly in this species. The crystals are uniform in size and are of the six-angled rhombohedral type. The line across the middle of the crystals is evident. The polarity of arrangement of the crystals with reference to the vascular bundles is very marked. The crystals on an average are larger than for *Cassia chamaecrista*, some attaining a size of 18 microns long and 13.5 microns wide (Plate LX, Fig. 9). The crystals are continued into the apex of the leaflets.

Pulvini. The regular six-sided prismatic crystals of the leaflets are also present in the pulvini, and are located in the endodermal region. There are relatively few of these crystals.

Petiole. Throughout the endodermis of the bundles an abundance of crystals is present, forming a continuous crystal sheath, with branches passing into the gland that is present on the dorsal side of the petiole (Plate LXV, Fig. 27). The crystals are very abundant in the lower half of the gland, being massed around the vascular bundle which enters the gland. No crystals are found in the terminal region of the gland. This upper portion of the gland resembles pulvinar structure. In the stem, the crystal distribution is like that in the petioles. The crystals are always present in the endodermis, and are found in all parts of the stem. Branches of crystal cells extend into the base of the cotyledons, but no crystals were observed in the cotyledons themselves. The crystal lines cease at the point in the main axis where the tap root begins. The writer was unable to find crystals in the roots of this plant.

The crystals of *Cassia nictitans* show the enveloping sacs better than any plant described thus far. The intercellular connecting threads are also very well brought out. By staining crystal cells in eosin for 48 hours, that had previously been fixed in chrom-acetic, and then destaining rather sharply, the writer was able to demonstrate very clearly the presence of intercellular connections. Good results were also obtained by following Gardiner's method to bring out intercellular connections. By this method the sections are put in iodine solution for 20 minutes, then in strong sulphuric acid for a few seconds; then stained deeply in aniline blue. After this process the crystals stand out clearly as white prisms in sharp contrast to the deep blue of the protoplasmic sacs. Between the adjoining crystal cells delicate striations are observed.

Mimosa albida

The large genus *Mimosa* was referred to before as including species that represent the climax of sensitivity in the Leguminosae. *Mimosa albida* (*M. sensitiva*) is native to the tropics of the New World like the great majority of the members of the genus. In the seedling stage, the first leaves bear 3 pairs of leaflets. Professor Macfarlane (Lecture Notes) demonstrated that the basal pair is less sensitive than the two terminal pairs. After 4 shock stimuli had been applied, the two terminal pairs closed, while the basal pair was closed to about half the extent of the terminals. The leaves that are developed later are bipinnately compound, with two leaflets to each leaf, and each leaflet bearing two pairs of secondary leaflets. The leaflets of the terminal pair are of equal size, and are obovate in shape. Of the basal pair, the outer is of the same shape, but slightly smaller than those of the terminal pair; the inner is very small and rudimentary. In the older leaves the small secondary leaflet disappears entirely. There is a well-defined primary pulvinus at the base of the petiole; secondary pulvini are present at the bases of the leaf midribs, and a tertiary pulvinus at the base of each secondary leaflet.

In the nyctitropic movement the terminal pair of secondary leaflets move upward back to back. The large basal secondary leaflet folds over the outer terminal pair; the small basal secondary leaflet also moves upward; the midribs converge and drop so as to make an angle of about 75 or 80 degrees with the petiole which also drops 40 or 45 degrees. This fully closed condition is observed only in the younger leaves. In the older leaves, the leaflets are closed ⅔ of their full extent.

The paraheliotropic movement of the leaves is in the same direction, and the same changes in position are noted as in the nyctitropic response. The movement begins in bright sunlight when the temperature reaches 29° C.

The leaves of *M. albida* are sensitive to mechanical, chemical and thermal stimuli. When a terminal secondary leaflet is stimulated, after a latent period of 1 to 1¼ seconds, it rises, followed very soon by its partner; in 28 seconds the basal pair moved upward, and in 3 seconds longer the midribs converged; in 8 seconds after that the basal pair of the other leaflet moved, followed by a partial closure of the terminal pair in 20 seconds longer. To cause complete closure of the leaflets 2 to 3 stimuli are necessary. The leaf drops 30 to 40 degrees. The re-expansion time of the leaves is 12 to 16 minutes. The small basal secondary leaflets show the same sensitivity as the larger ones, for the same sequence follows when one of them is stimulated, as when its large partner is stimulated. The propagation of stimuli through the stem of this plant is rather limited. If a leaf be stimulated, as described above, the stimulus may be carried to the next leaf above, or occasionally to two leaves in a young shoot. Chemical stimuli in the form of acids, alkalies and alcohol cause a closure of the leaflets. Closure is effected also when heat is applied, or when ice is placed on the leaflets.

Leaflets. The crystals along the veins are mostly of the six-angled prismatic type, but there are also quadrangular prisms present. The lines are fairly regular, but occasionally gaps are noted in the continuity of the crystal cells. Usually a crystal fills ½ to ⅔ of the lumen of the cell in which it is contained. A fairly diffuse protoplasmic sac envelops each crystal.

Pulvini. No crystals were observed in any of the pulvini. This is the first of the sensitives described so far in which no crystals, neither prisms nor conglomerates, are present. The structural details of the pulvini resemble very closely those for *Mimosa pudica.*

Petiole and Stem. Continuous crystal lines extend through petiole and stem in the endodermis. The crystals are mostly the six-angled rhombohedral type, or the quadrangular type, very much the same relation as in the leaflets (Plate LX, Fig. 10). Occasionally an elongated, rather narrow, styloid-like crystal is observed in the lines of crystals. This form is of the type already noted in *Robinia* and *Pueraria*, and as noted for those plants, has projecting processes at the ends of the crystals. It is noteworthy that all three forms of crystal, in some of the individual crystals, show the partition across the middle. This would indicate that they are in all probability twin crystals. The occasional presence of two crystals in the same cell, as noted before, further points to the same conclusion.

Mimosa pudica

There is probably no sensitive plant that has been observed for so long a time and has been studied more extensively than *Mimosa pudica*—the Sensitive Plant. It is of good size, with quite large bi-pinnately compound leaves that show irrito-contractility to a high degree. On account of the very striking sensitive movement of the leaves the plant has long been a favorite object for the study of irritable responses. The relative ease of growing this plant in greenhouses in different climates has been another helpful factor in making it a world wide favorite for laboratory purposes. The leaves are highly sensitive to nyctitropic, paraheliotropic, mechanical, chemical and other forms of stimulation. The direction of movement and the position after contraction is the same for the different kinds of stimuli just given. That is in all cases of stimulation the secondary leaflets move upward and forward, the midribs converge and the whole leaf drops. Such complete closure, to be sure, takes place only under optimum environment.

Cotyledons. The cotyledons of *Mimosa pudica* show sensitive movement when they are quite young—3 to 8 days old. When older they become yellowish and lose all sensitivity. Darwin (11, p. 127) considers the cotyledons of this plant to be very feebly sensitive. Macfarlane (31, p. 203) states that the cotyledons are markedly sensitive. "The cotyledons of the Sensitive Plant are most active during the period that their activity is of greatest benefit, viz. in the very young state (seedlings 2 to 10 days old), since their great function is to protect the first leaves and the growing bud. When the latter have pushed out above the cotyledonary tips, the protective function has ceased, and their irritable movements are greatly lost." Two to three stimuli are required to cause the cotyledons to rise through an angle of 80 degrees. The movement is rather slow, resembling the movement of the leaves of less sensitive plants, like some of the Desmodiums.

Large conglomerate crystals are scattered through the mesophyll of the cotyledons. No prismatic crystals are present. The pulvini of the cotyledons are devoid of crystals.

Leaflets. The crystals are mostly of the hexagonal type, with relatively few of the quadrangular forms. The crystal cells are continuous along the veins, forming close sheaths on the ventral side of the vascular bundles. The crystals are large, usually filling ⅔ to ¾ of the cell cavities. Very often the crystals touch the dividing cell walls.

Pulvini. No crystals are present in the pulvini.

Petiole, Stem and Root. A continuous system of crystal cells, which form sheaths round the bundles, is present in the petioles and in the stem of *Mimosa pudica*, with breaks only in the continuity in the pulvini. The regular crystals are also present in the sensitive lanceolate stipules. In the stem the crystal cells become more diffuse near the beginning of the primary root, and are entirely wanting in the root endodermis.

The regularity in the shape of the individual crystals, the extensive distribution of crystals throughout the plant, the distinct protoplasmic sheath around each crystal, intercellular connections between the crystal cells, contact of some of the crystals with the dividing cell walls, all these show a decided advance in crystal relation over the plant previously described.

Mimosa Spegazzini

Of the species of *Mimosa* that have been studied, *M. Spegazzini* shows the most highly specialized irritability. It is slightly more sensitive than *Mimosa pudica* in that the different phases of a stimulation act are slightly shorter than for the latter species. Twenty to thirty pairs of secondary leaflets are borne by each of the two leaflets. Primary, secondary and tertiary pulvini are well developed. Rather strong hairs are developed on the entire plant.

The nyctitropic movement of the leaves is very similar to that of *Mimosa pudica.* The secondary leaflets move upward and forward, the midribs converge and the whole leaf drops about 90 degrees (Plate LXI, Fig. 12 and 13).

When a mechanical stimulus, such as a forceps pinch, is applied to one of the terminal secondary leaflets after a latent period of less than ¼ second the leaflet stimulated rises and its partner almost at the same time. The stimulus is then carried down the midrib, the pairs of secondary leaflets closing in order; in 9 seconds all the secondary leaflets have closed, the midribs converge followed in 3 seconds by a drop of the entire leaf. The stimulus moves up the other leaflet with the result that the secondary leaflets close in order. In 20 seconds after the stimulus had been applied all the secondary leaflets are closed. The stimulus is propagated through the stem to other leaves. The writer in one case observed that five leaves above the one stimulated and three below dropped. The extent of propagation of stimuli through the stem is a decided advance from the irrito-contractile view point over that of *Mimosa pudica.*

In crystal distribution the relation in *M. Spegazzini* is almost exactly parallel with that of *M. pudica.* The crystals are of the same type, of the same relative size, are present in leaflets, stipules, petioles and stem, but not in the pulvini. The chief difference between the two species, in regard to the crystals, is that in *M. Spegazzini* 5 to 7 lines of crystals pass into the bases of the strong, stiff hairs that are scattered over both surfaces of the leaflets. Are these hairs irritable? The writer was unable to prove conclusively that the hairs are irritable centers. Haberlandt (19) states that the hairs of *Biophytum* are sensitive.

The distinct intercellular connections of the crystal cells can be clearly demonstrated in *M. Spegazzini*. By staining such cells very deeply, followed by destaining, the threads connecting the adjacent cells stand out quite clearly (Plate LX, Fig. 11).

OXALIDACEAE

As in the case of Leguminosae, the writer studied the histological details of certain species of this family, including forms that are native and others that have been introduced from the tropics, and are grown in greenhouses.

Oxalis stricta

This common native species exhibits very interesting sensitive movements of its leaflets. The nyctitropic movements were carefully studied by Ulrich (45, p. 226). The same author also noted the paraheliotropic response of the leaflets. Macfarlane (30, p. 189) describes the behavior of the leaflets when mechanical stimuli are applied. "After a sharp but delicate mechanical stimulus applied with a pencil or other instrument to a terminal leaflet, a latent period of 3⅛ seconds elapses, followed by a period of slow but gradually accelerating contraction during the next 4 seconds. From the 7 to the 20th second the motion is rapid, but thereafter slows down gradually to the 30th second and then becomes increasingly slow till the 45th second when the contraction ceases. After 15-18 minutes expansion begins, and a very slow rise can be traced till the leaf regains its expanded state in 45-50 minutes."

Leaflets. In the leaflets small crystals are distributed through the mesophyll tissue. These vary in shape from some that are somewhat irregular, approaching a conglomerate form, to others that are prismatic —four to eight-angled usually.

Pulvini. In the secondary pulvini no crystals are present. In the primary pulvinus few large conglomerates are found scattered in the cortical zone.

Petiole. Large crystals, arranged in irregular lines, are present in the cortex just around the vascular bundles. Some of the crystals are 40 microns in diameter, and lie in very large cells. In shape the crystals might be described as being intermediate between conglomerates and quadrangular prisms. The same type is represented for *O. Ortgiesi* (Plate LXIV, Fig. 21).

Oxalis violacea

This specimen was compared with *Oxalis stricta* in its nyctitropic movements and the crystal relation. The movement of the leaflets in night sleep is exactly like that of *O. stricta.*

The crystals scattered throughout the leaflet tissue are more numerous than in *O. stricta* and are more varied in shape. Some of the crystals are long and narrow, 38 microns long and 8.5 microns wide.

Oxalis corniculata

In this, another very common native species, the crystals in the leaflets are more numerous and more regular in shape than in either of the two species described. Most of them are prisms, or tetrahedra fewer are of the conglomerate type. The crystal relation approaches very closely that of the next species.

Oxalis floribunda

This introduced species is native to the mountainous regions of Chili. The three obcordate leaflets of each leaf show nyctitropic movement (Plate LXII, Fig. 14-15). The leaflets are very feebly sensitive to mechanical stimuli.

The crystals are numerous, vary in shape but are mostly prismatic, and are scattered through the mesophyll tissue (Plate LXIV, Fig. 23). A few of the crystals are elongated with a partition line across the middle, and in general structure resemble the styloid-like crystals described for the Leguminosae.

Oxalis Ortgiesi

This is another South American species, being indigenous to Peru. The leaflets exhibit night sleep.

The leaflets also respond to mechanical stimuli, but show slow, rather sluggish movement.

The type of crystal present in the leaflets is similar to that of *O. floribunda.* In the petioles and stem large crystals are found, which in shape approach a prismatic form. These are scattered through the cortex but more abundantly near the bundles (Plate LXIV, Fig 21).

Oxalis bupleurifolia

Of the different species of *Oxalis* examined, this is the most sensitive in its leaflets. Each leaf consists of a rather long, flattened petiole a quarter of an inch wide, that bears three small delicate obovate leaflets. In nyctitropic movements, the petiole, as well as the leaflets, occupy different positions from those of the day positions. Darwin (11, p. 328) notes the movement of the petiole. "The foliaceous petiole rose during the day and early part of the night, and fell during the remainder of the night and early morning." He also observed the movement of the leaflets. Ulrich (45, p. 220) gives results obtained on stimulating the leaflets with mechanical and with electrical stimuli. "When stimulated mechanically the leaflet dropped in 0.75 seconds at a temperature of 30° C. Eleven subsequent stimulations were given of equal intensity a minute apart; each caused the leaf to fall slightly lower than the preceding one. The leaflet had entirely recovered in thirty minutes from the begining of stimulation."

Leaflets. Numerous huge, somewhat irregular crystals are scattered through the mesophyll of the leaflets, with more of these toward the lower surfaces. The crystals resemble very closely those found in *O. Ortgiesi*, but exhibit greater regularity and uniformity in their shapes. (Plate LXIV, Fig 22). Each crystal is 32-36 microns in diameter.

Pulvini. The type of crystal found in the leaflet is continued into the small secondary pulvini, but here relatively few crystals are found. In the primary pulvinus a greater number of the large crystals are present scattered through the cortex.

Petiole. The foliaceous petiole of this plant shows a very interesting crystal development, with one to three continuous lines of regular crystals along the ventral side of each of the numerous bundle strands extending through the petiole. In shape the crystals resemble the six-angled rhombohedral type so characteristic of the more sensitive members of the Leguminosae, but the angles are not as sharp as those of legumes, and the individual crystal is somewhat barrel-shaped. The line across the widest part of the crystal is very evident in most of the crystals. Each crystal occupies ⅓-⅔ of the crystal containing cell, and is enveloped in a rather diffuse protoplasmic sac.

Of the different species of *Oxalis* that were examined the relation in the petiole of *O. bupleurifolia* represents the highest development of crystals, that is not including the species of *Biophytum* which by some writers are included in the genus *Oxalis*.

Biophytum dendroides

The genus *Biophytum* includes some 20 species scattered through the tropics of both hemispheres. *B. dendroides*, indigenous to Brazil and Peru, is readily grown in greenhouses where it flowers and fruits very freely. Fifteen to twenty-five pairs of oblong leaflets are borne on each of the pinnately compound leaves. A short, rather thick secondary pulvinus is present at the base of each leaflet, and a well defined primary pulvinus at the base of the petiole. A similar pulvinus is found at the base of each of the long flower stalks.

The night sleep of this plant is very striking. The leaflets drop downward through an angle of 85-90 degrees, and the whole leaf falls through 15-20 degrees. The upright flower stalks drop 70-80 degrees (Plate LXIII, Fig. 16-17). The paraheliotropic response is equally pronounced when the temperature, in bright light, reaches 29-30° C.

The leaflets are likewise quite highly sensitive to mechanical, chemical, thermal and electrical stimuli. Macfarlane (30 p. 194-195) describes fully the effects of the first three of these forms of stimulation. When a terminal leaflet is stimulated mechanically it and its neighbor fall through an angle of 40-45 degrees, after a latent period of ¾ second to 2 seconds, varying with the relative age of the leaflets. The stimulus is propagated through the midrib, the pairs closing in regular succession toward the base, with a time interval of 2½ seconds between each pair. If a second stimulus is applied, the leaflets will fall through 20-25 degrees, and on the application of a third stimulus they fall through a small angle. A fourth and even a fifth stimulus may cause a slight additional drop. Summation action is here necessary to cause closure of the leaflets."

Macfarlane (30 p. 196) proved very conclusively that in *Oxalis dendroides*, as in other sensitives, a stimulus is propagated more rapidly in a centrifugal, than in a centripetal direction. "A particle of ice placed on the pulvini of the middle pair, i. e., the tenth if there 19 pairs, will excite all the pairs above it within 15-17 seconds, but 21-23 seconds will elapse before the lowest pair in such a leaf as the tenth from the apex-bud closes."

Leaflets. The crystals here are confined only to the endodermal region of the vascular bundles, being arranged in fairly continuous lines. In shape, the crystals resemble those of the Mimosas, but are less uniform in size and more irregular in shape than in the Mimosas. Many of the crystals are six-angled and indicate a line across the middle. (Plate LXIV, Fig. 20).

In general, the long axes of the crystals are parallel to the direction of the vascular bundles, but occasionally the crystals lie cross-wise in the crystal cells.

Pulvini. In both primary pulvinus, and secondary pulvini, a few large scattered crystals occur in the cortex. These are quite irregular and do not resemble the type of crystal present in the leaf.

Petiole. The lines of crystal cells in the petiole are of the same nature as those along the veins of the leaflets. Each crystal is imbedded in a protoplasmic sac.

Biophytum sensitivum

This species of *Biophytum* is another fairly common green house plant, that is native to the Tropics of both the Old and the New World. In structural details and in leaf sensitivity it resembles rather closely *B. dendroides*, but is slightly more sensitive than the latter.

The morphological characters of the plant, and the day and night positions of the leaflets, are indicated in the illustration (Plate LXIII, Fig. 18-19).

Leaflets. As in the preceding species, continuous lines of crystal cells are associated with the vascular bundles of the leaflets. The majority of crystals are of the regular, six-angled, rhombohedral type. Here and there a barrel-shaped crystal is noted in the lines of crystals. In the fully developed leaflets, each crystal fills ⅔ of the lumen of the cell in which it is contained (Plate LXIV, Fig. 24-25). While the leaflets are small, immature and are non-chlorophylloid, or are very pale green, the crystals are relatively small. It is noteworthy that at this stage the leaflets are non-sensitive or very feebly sensitive. Cunningham (10) showed that in *Mimosa pudica* the leaflets do not attain their maximum sensitivity until they have developed the characteristic deep green color.

Pulvini. The lines of crystal cells end at the beginning of the pulvini.

Petiole. The crystals are of the same type as in the eaflets, and form crystal sheaths along the vascular bundles. Each crystal is enveloped in a protoplasmic sac, with fine connecting intercellular threads between the adjacent crystal cells.

Nature of the Crystals

The crystals present in the two families studied—Leguminosae and Oxalidaceae—consist of calcium oxalate, as was proved by applying various chemical tests. Very thin fresh sections were used in the tests. It is important to use thin sections so as to have the crystals exposed as much as possible to the chemical reagents that are applied. To such preparations chemicals were added and the results observed carefully under the microscope.

When concentrated hydrochloric acid is added to sections containing crystals, it is noted that the crystals dissolve gradually. Conglomerate crystals dissolve more readily than the prismatic forms. In the solution process the conglomerates are broken up into the small prisms of which each crystal is composed, and these particles are then dissolved. Each of the rhombohedral crystals in the solution action of the acid is divided into two segments, the division taking place along the partition line across the middle of the crystal. This seems to indicate that this line is different in resistance to the rest of the crystal, and may even consist of a different substance. Complete solution of the crystals usually takes place in 1-3 minutes. Crystals separated from the mucilaginous sheaths in which they are enveloped are dissolved in a shorter time, the sheaths retarding the solution action.

Concentrated nitric acid acts like concentrated hydrochloric but dissolves the crystals in a slightly shorter time. The crystals are likewise soluble in concentrated sulphuric acid, concentrated caustic potash, but insoluble in acetic acid.

Aggregation

Darwin (12, p. 38) in his extensive studies on *Drosera rotundifolia* observed that a very interesting change takes place in the appearance of the contents of tentacular cells when stimuli are applied to the irrito-contractile tentacles. "If the tentacle of a young, yet fully matured, leaf that has never been excited or become inflected be examined, the cells forming the pedicels are seen to be filled with homogeneous, purple fluid. The walls are lined by a layer of colourless circulating protoplasm. If a tentacle is examined some hours after the gland has been excited by repeated touches, or by an inorganic or organic particle placed on it, or by the absorption of certain fluids, it presents a wholly changed appearance. The cells, instead of being filled with homogeneous purple fluid, now contain variously shaped masses of purple matter, suspended in a colourless or almost colourless fluid." Darwin described the phenomenon as an aggregation process, and the masses formed are "aggregation bodies." The shapes of these masses are fully described in Darwin's "Insectivorous Plants".

De Vries (14, p. 6) made additional researches on the aggregation phenomena in *Drosera rotundifolia*, and distinguished three phases in agregation—(1) Retarded and much more clearly differentiated circulation of the peripheral protoplasm. (2) Division of the vacuole into numerous small vacuoles which all remain surrounded by a part of the wall of the original vacuole. (3) A very significant diminution of the volumes of the vacuoles.

Bokorny (3, p. 427-473) who studied aggregation in a number of plants, not including any of the sensitives, considered the aggregation mass to be a proteid which separates from the protoplasm. As to the biological significance of aggregation, this author ventures no explanation.

Loew and Bokorny (26, p. 614) refer to aggregation as an "echte Lebensreaction".

Klemm (22, p. 395-420) studied the nature of the substances that appear in the aggregation masses. Among the constituents are proteins, fats and occasionally tannin.

The irrito-contractile centers, especially the pulvini, of most of the sensitives taken up in this investigation, were examined for aggregation. Each pulvinus in its structure, as fully described by Sachs (40, p. 793) shows a central vascular bundle cylinder, around which a wide cortex is present, the width of the cortex varying in the different sensitives. It is noteworthy that the structure of the pulvini is very similar in both Leguminosae and Oxalidaceae.

If the pulvini are put into a strong fixing solution of iron or gold or platinum chloride while the leaflets are expanded, the cortical cells of the pulvini are granular in their contents, but indicate no definite masses. It should be observed that it is difficult to fix pulvinar tissue without getting aggregation in that the cutting of the leaflets to put into the fixing solution acts as a stimulation and causes aggregation.

When sections, taken from pulvini of such plants as *Mimosa pudica* or *Biophytum sensitivum*, are examined under the microscope they indicate rather clear shining globular masses in most of the cells of the cortex. These masses occur throughout the entire cortex, but relatively more abundantly on the ventral side of the pulvinus, that is they are more abundant on the side away from the light. Each contracted aggregation mass occupies ⅖-½ of the cavity of the cell.

The aggregation masses can be studied to better advantage if certain chemicals are applied. For this purpose the writer has found the following to be helpful—gold chloride, platinic chloride, ferric chloride, ammonium carbonate, caffeine, silver chloride and silver nitrate. Gold chloride when applied in a 5% solution stains the masses a purplish brown.

Aggregation bodies in the pulvini of the various sensitives become relatively more abundant, are larger and more uniform in size as one passes from the less sensitive to the highly sensitive plants. In such plants as *Oxalis corniculata*, *O. floribunda* and *Amphicarpaea monoica* aggregation is not as pronounced as in more specialized plants like the Biophytums and the Mimosas. *Cassia chamaecrista* represents a good intermediate type. In the Cassias aggregation is observed in the cells of the terminal portion of the petiolar gland as well as in the cells of the pulvini. In feebly sensitive plants like *Gleditschia* or species of *Oxalis* when sections of the pulvini are treated with, e.g., gold chloride, or ferric chloride, it is observed that the aggregation bodies form slowly, being small at first, and are often irregular, such as Darwin noted in *Drosera*. Very often a number of small masses may be present in the same cell. These smaller masses may coalesce and form a large body. In the highly sensitive plants the aggregation masses form almost instantly when treated with the various chemicals mentioned above. *Nature of the Aggregation Bodies*, various chemical tests were used as to the to determine if possible the nature of the aggregation substance. These tests indicate that the masses are proteinaceous in nature. The xanthoproteic test gives a positive protein test. Osmic acid stains the masses a grayish-black, changing to a deep black. This suggests that a fat or lipoid body may be present in the aggregation masses, but other characteristic tests for fats, such as staining with alkannin gave negative results.

The aggregation masses are readily soluble in 95% alcohol, concentrated caustic potash, and glacial acetic acid, but are resistant to concentrated nitric and concentrated sulphuric acids.

SUMMARY OF RESULT

1. In geographical distribution it is shown that sensitive plants are very rare in cold temperate regions, become more abundant in warm temperate and sub-tropical regions, and attain their climax of development alike in number of species and high degree of sensitivity, in the tropics.

2. The majority of the highly sensitive species are natives of subtropical and tropical America, from the Southern States, through Mexico, Central America, tropical and sub-tropical South America.

3. The most wide spread irritable response shown by these is the nyctitropic, or so-called sleep movement, which has in all probability been induced by rapid changes in temperature, transpiration and radiation effect in transition from day to night.

4. The paraheliotropic response probably next succeeded, while response to light stimuli, and to mechanical stimuli next became pronounced.

5. Under experimentation, equally marked responses to chemical, thermal and electrical stimuli are observed.

6. In relation to previous studies and discussions on propagation of stimuli, the writer advances the view that this phenomenon is centered in the endodermis. The cells of this layer contain a greater or less number of crystals of oxalate of lime, which in number and in progressive perfection of development show transition from less sensitive to the most sensitive types.

7. Amongst the less sensitive plants, the crystals are often conglomerates, irregularly scattered outside the endodermis, or in discontinuous lines along the endodermis and surrounding the bundle.

8. With increasing sensitivity and advance toward tropical conditions the crystals show, alike in the Leguminosae and the Oxalidaceae, similar progressive advances in abundance of crystals, regularity of shape in these, and restriction of them to the endodermis.

9. The climax in this process is seen in *Mimosa pudica* and *M. Spegazzini* amongst the Leguminosae, and in *Biophytum sensitivum* and *B. dendroides* in the Oxalidaceae. These represent also the most sensitive plants of the two families.

10. In histological distribution the crystals and crystal cells of the endodermis become in the most sensitive species continuous from the leaf margins and even in *M. Spegazzini* from the bases of the hairs, along all of the veins of the leaflets, the petiole and the stem. But in the irrito-contractile pulvinus regions, that receive and respond to stimuli, the crystals are absent. In less sensitive pulvini, however, conglomerate and transition crystals toward the most perfect type are present.

11. Each crystal cell in the higher types contains a rhombohedral crystal that is traversed across its middle by a cleavage plane, that is first affected by solvents such as concentrated hydrochloric and nitric acids.

12. Surrounding each crystal is a protoplasmic sac that stains deeply with protoplasmic stains, while from the sac intercellular uniting threads seem to pass through the common membranes between the adjacent cells, so as to form continuous protoplasmic connections throughout the endodermal tissue.

13. The writer would view the crystals and the continuous protoplasmic investments and connections between these as the special conducting lines for the passage of stimuli.

14. With transition from the less sensitive to the more sensitive species the cells of the pulvini contain, in increasing amount and complexity, aggregation bodies resembling those previously described by Darwin and other investigators, as associated with irrito-contractile centers.

15. These masses show contraction and aggregation changes under stimulation.

16. From varied chemical reactions (tests for various cell contents) the writer would view the aggregation bodies as being proteinaceous in nature.

17. All irrito-contractile changes, alike during contraction and expansion, seem to be due to changes primarily in the protoplasmic sac surrounding each aggregation body, next in the aggregation body itself, finally in the amount of liquid these may absorb or give off.

18. In fixation, demonstration and identification of the aggregation masses the most helpful chemical agents were found to be gold chloride, platinic chloride, ferric chloride, ammonium carbonate, silver chloride and silver nitrate, the first two giving a brownish purple to purple color to the aggregation bodies.

19. The writer agrees with Haberlandt in considering that the complex hairs, often found over the irrito-contractile pulvini, or even over the general leaflet surface, seem to act as delicate receptors of environal stimuli.

20. Among the varied types examined the two most specialized hairs are those distributed over the leaflets of *Desmodium canescens* and *Mimosa Spegazzini*, in the former of which special bundle diverticula pass into the bases of the hairs; in the latter prolongations, from the zone of crystal cells rise up into the bases of the hairs.

21. All present evidence seems to point to the conclusion that, alike in seedling and adult axes, crystal cells and aggregation masses gradually become fewer in the hypocotyl and are entirely absent in the root.

22. For all of the above sensitive plants now studied, as for *Drosera* recorded by Darwin, and *Dionaea* recorded by Macfarlane, all forms of energy—thermal, luminous, chemical, mechanical, electrical and molar stimuli, can act as stimulants to produce irrito-contractile movements.

Explanation of Plates

Plate LVIII

Fig. 1. *Cassia nictitans* showing the expanded day position of the leaves.

Fig. 2. *Cassia nictitans.* Leaves in nyctitropic position. The leaves have dropped and the leaflets are folded upward.

Plate LIX

Fig. 3. Section of leaflet of *Pueraria Thunbergiana.* Styloid type of crystal along veins and in mesophyll.

Fig. 4. T. S. secondary pulvinus of leaflet of *Pueraria Thunbergiana* crystals around vascular bundle cylinder and in cortex.

Fig. 5. Section of leaf of *Bauhinia diphylla.* Scattered conglomerate crystals and discontinuous lines of prismatic crystals along the veins.

Plate LX

Fig. 6. L. S. secondary pulvinus of Gymnocladus leaflet of *Gymnocladus disica.* Conglomerate crystals scattered through the cortex and a broken line of prismatic quadrangular crystals along the vascular bundle.

Fig. 7. L. S. petiole of leaf of *Robinia Pseudacacia* showing elongated styloid and prismatic types of crystals.

Fig. 8. Surface section leaflet of *Desmodium canescens.* Lines of crystals along the veins.

Fig. 9. Macerated leaflet of *Cassia nictitans.* Lines of crystals along the veins.

Fig. 10. L. S. petiole of leaf of *Cassia nictitans.* Crystals are mostly of the six-angled rhombohedral type; others are quadrangular and a few styloid.

Fig. 11 Section of leaflet of *Mimosa Spegazzini.* Lines of crystal cells. Crystals are regular six-angled and enveloped in distinct protoplasmic sheaths.

Plate LXI

Fig. 12. *Mimosa Spegazzini.* Expanded day position of the leaves.

Fig. 13. *Mimosa Spegazzini.* Nyctitropic position of the leaves.

Plate LXII

Fig. 14. *Oxalis floribunda.* Day position of the leaves.

Fig. 15. *Oxalis floribunda.* Nyctitropic position of the leaves.

Plate LXIII

Fig. 16. *Biophytum dendroides.* Day position of the leaves.

Fig. 17. *Biophytum dendroides.* Nyctitropic position of the leaves.

Fig. 18. *Biophytum sensitivum.* Day position of leaves.

Fig. 19. *Biophytum sensitivum.* Nyctitropic position of leaves.

Plate LXIV

Fig. 20. Section, leaflet of *Biophytum dendroides.* Many of the crystals are six-angled and indicate a line across the middle.

Fig. 21. L. S. stem *Oxalis Ortgiesi.* Crystals scattered in cortex are intermediate between conglomerates and quadrangular prisms.

Fig. 22. Section leaflet *Oxalis bupleurifolia.* Crystals in mesophyll and along veins-

Fig. 23. Section of leaflet *Oxalis floribunda.* Small crystals scattered through mesophyll tissue.

Fig. 24. Section leaflet *Biophytum sensitivum.* Lines of large hexagonal prismatic crystals.

Fig. 25. Surface view younger leaflet *Biophytum sensitivum.* Crystals relatively small.

PLATE LXV

Fig. 26. T. S. petiole and gland from leaf of *Cassia chamaecrista.* Aggregation material in the gland.

Fig. 27. L. S. petiole and gland of *Cassia nictitans.* Lines of crystals in petiole and in basal two-thirds of gland.

Fig. 28. T. S. secondary pulvinus of leaflet *Amphicarpaea monoica.* Relatively few aggregation bodies.

Fig. 29. T. S. secondary pulvinus, *Mimosa Spegazzini.* Aggregation bodies numerous.

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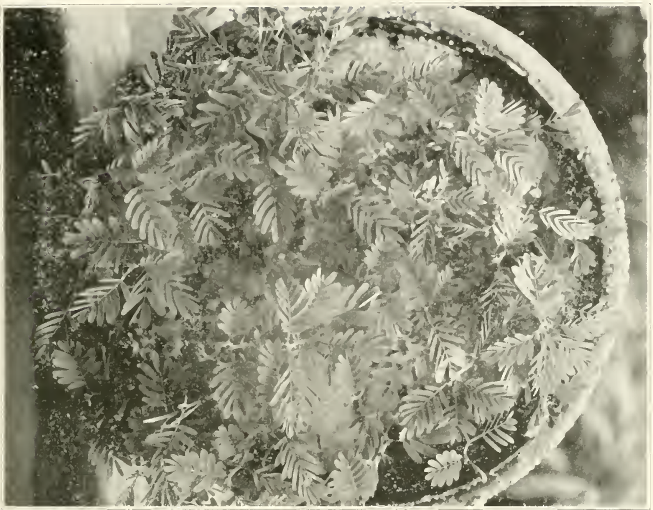

FIG. 1

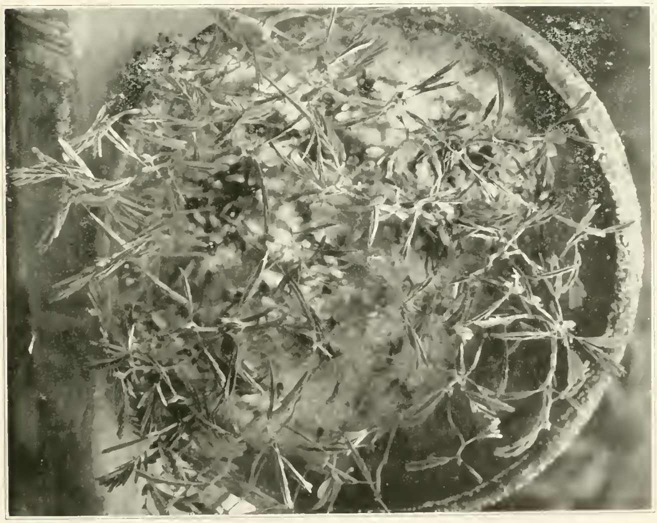

FIG. 2

STECKBECK ON SENSITIVE PLANTS

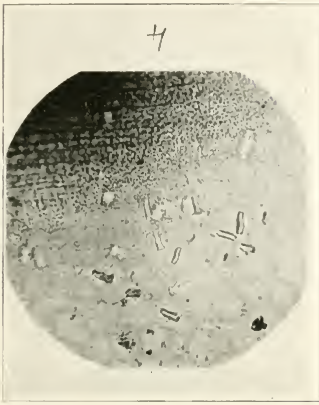

FIG. 3

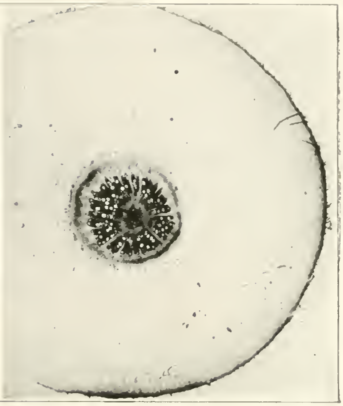

FIG. 4

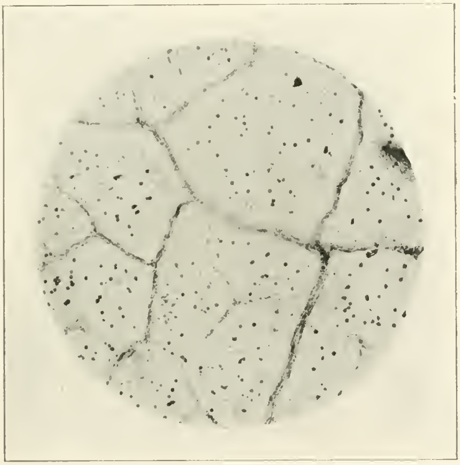

FIG. 5

STECKBECK ON SENSITIVE PLANTS

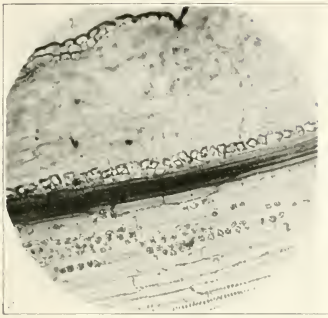
FIG. 6

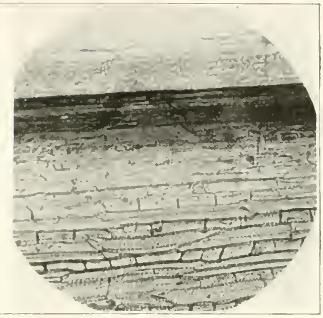
FIG. 7

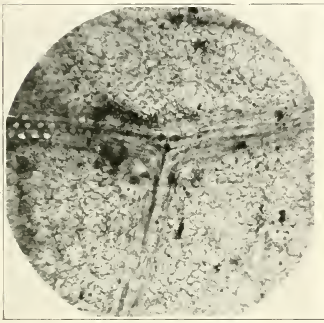
FIG. 8

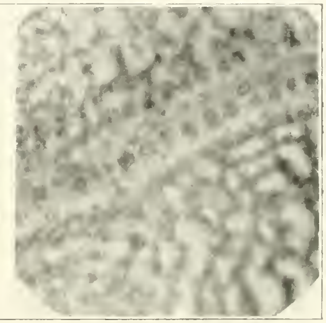
FIG. 9

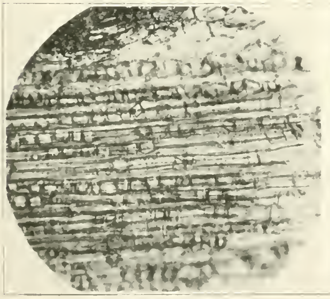
FIG. 10

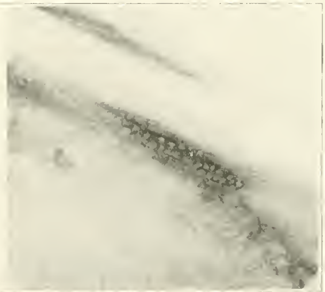
FIG. 11

STECKBECK ON SENSITIVE PLANTS

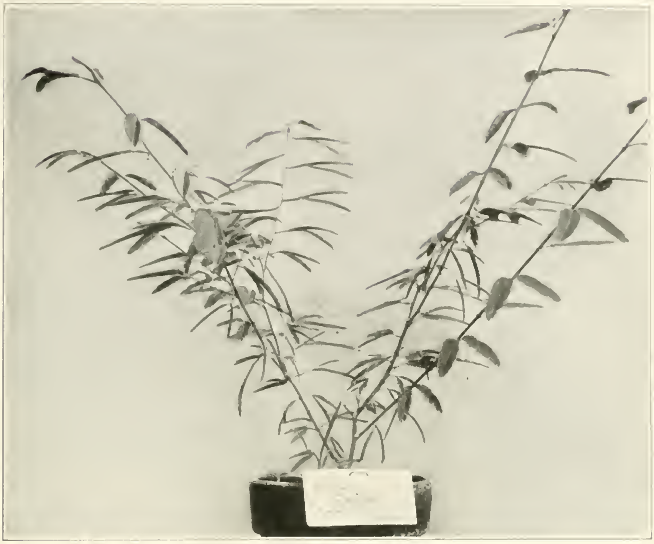

Fig. 12

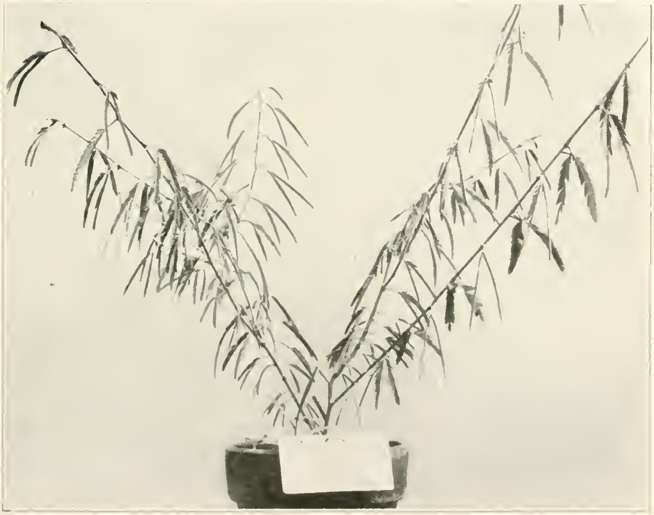

Fig. 13

STECKBECK ON SENSITIVE PLANTS

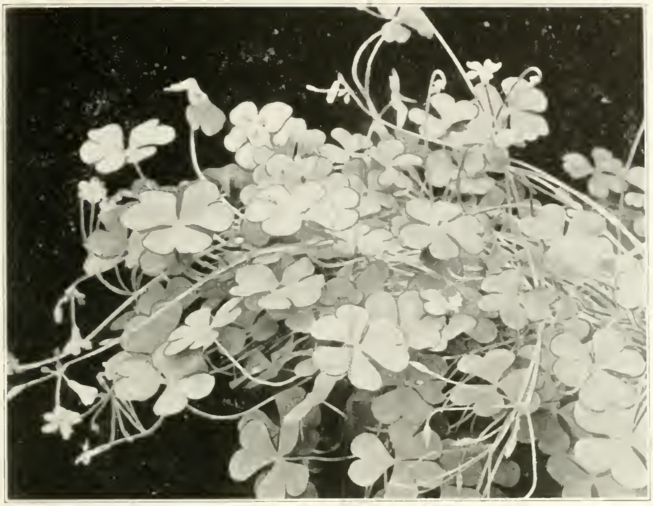

FIG. 14

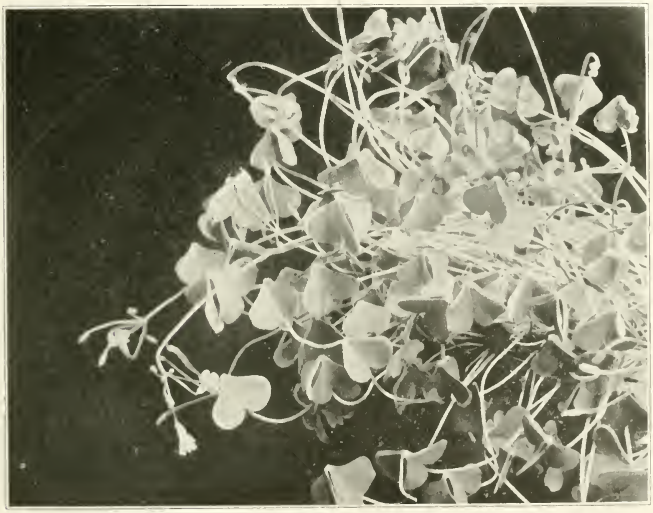

FIG. 15

STECKBECK ON SENSITIVE PLANTS

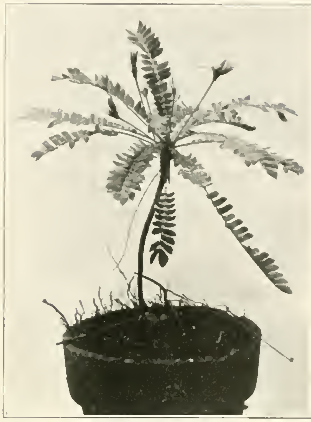

FIG. 16

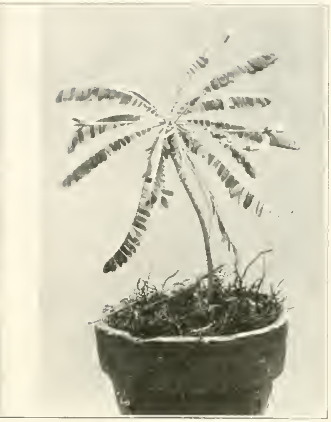

FIG. 17

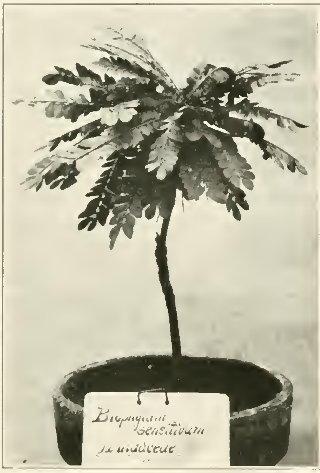

FIG. 18

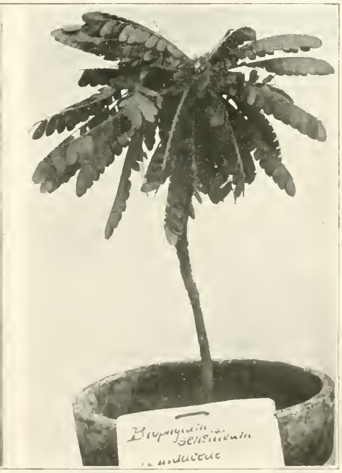

FIG. 19

STECKBECK ON SENSITIVE PLANTS

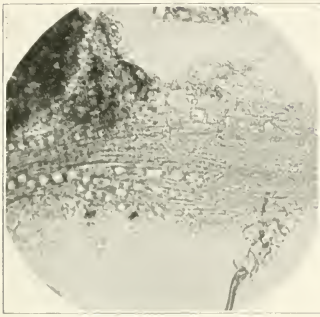

Fig. 20

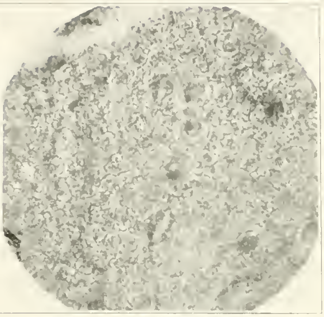

Fig. 21

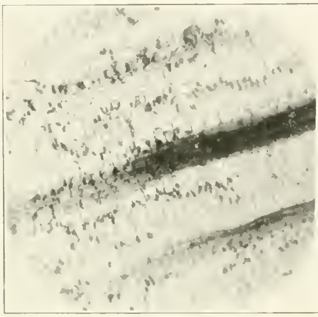

Fig. 22

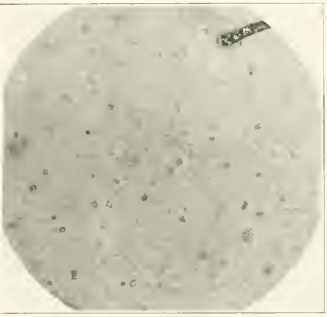

Fig. 23

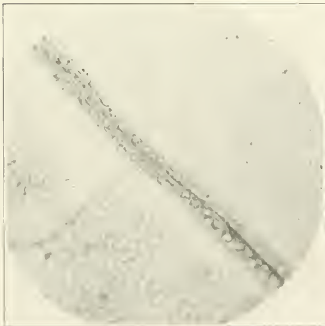

Fig. 24

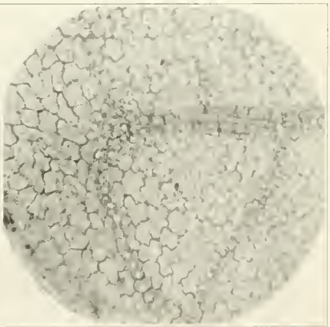

Fig. 25

STECKBECK ON SENSITIVE PLANTS

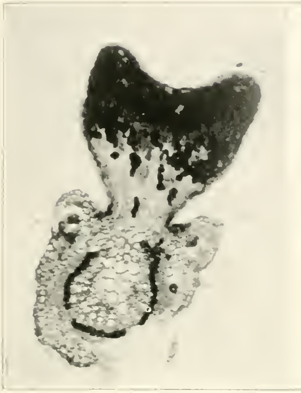

Fig. 26

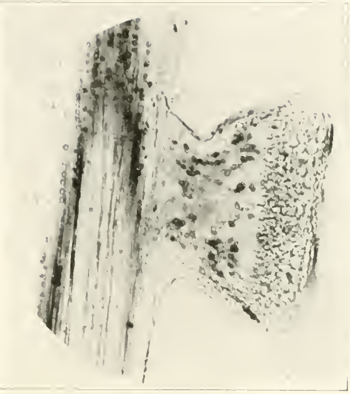

Fig. 27

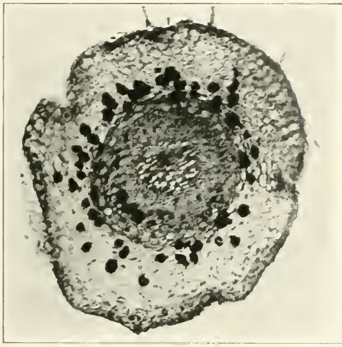

Fig. 28

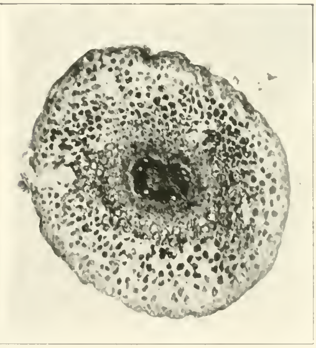

Fig. 29

STECKBECK ON SENSITIVE PLANTS

OBSERVATIONS ON THE BEACH PLUM
A STUDY IN PLANT VARIATION

BY

John Young Pennypacker, A.M., Ph.D.

With Plates LXVI-LXX

(Thesis presented to the Faculty of the Graduate School
of the University of Pennsylvania, May 1915,
in partial fulfilment of the requirement for
the Degree of Doctor of Philosophy).

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I. Introduction and History

1. *Introduction*

This subject was first suggested to me by Dr. J. M. Macfarlane, he having pointed out to me the marked variations which he had observed in the fruits of the plants which are produced at various places along the Atlantic coast from Cape Cod, Mass., to Cape May Point, N. J., and upon which he later published a paper entitled, "The Beach Plum, Viewed from Botanical and Economic Aspects" (18, p. 216). In that paper, he dwelt entirely on the variations as exhibited by the fruits, and also suggested various evolutionary lines by which these varieties may have evolved. He suggested to me, in view of the facts just set forth, the possibility of finding variations in other aspects of the plants, which I might correlate with the fruit variations. It was with this idea in mind that I undertook the present work. After extensive research, I have found certain varietal differences, which seem of sufficient importance to warrant the division of *Prunus maritima* (Marsh) into the following varieties:—

1. *Prunus maritima* var. caerulea-magna.
2. " " var. caerulea parva.
3. " " var. praecox.
4. " " var. purpurea magna.
5. " " var. purpurea parva.
6. " " var. rubra magna.
7. " " var. rubra parva.
8. " " var. lutea magna.
9. " " var. lutea parva.

2. *Review of Literature*

The history of the work which has been done upon this plant deals almost entirely with the taxonomic or systematic phase of the species. At first it was described by different botanists under numerous synonyms, but the similarity of these being later discovered it is now known as *Prunus maritima*.

It was first described by Marshall (19, p. 112) in 1785. He called it Prunus maritima, "The Seaside Plumb," and the description, though brief, was probably sufficient for that day. By way of general interest and comparison, it is now quoted: "This grows naturally toward the sea coast, rising to the height of eight to ten feet, often leaning and spreading into many branches. The leaves are oblong, rather smaller and not so pointed as those of the common plumb; smooth and of a shining green on the upper surface, but something lighter underneath, and slightly sawed on the edges. It is generally well filled with flowers, a few of which are succeeded by small, roundish fruit."

The authority usually given as the first describer is Wangenheim (31, p. 103) in 1781. A copy of this work from the Academy of Natural Sciences of Philadelphia was carefully searched but no record of *Prunus maritima* was found. In another work (32, p. 103) published in 1787, Wangenheim describes this species. Through some error in the dates of these publications, which has been passed on without investigation, it seems that Marshall (19, p. 112) has never been given credit for what he truly deserves, namely—the original describer of *P. maritima.* On the title page of Wangenheim (31) I found that he served as a captain in the Hessian Army during the Revolutionary War from 1777-1780. According to Stone (27, p. 491) this specimen which Wangenheim described came from Long Island, N. Y. In view of these facts, and since there is no mention of the name of the collector given by Stone (27), at the above named place, it seems probable that he saw this plant, while in the army service, and upon his subsequent return to Germany published what has been considered the original description.[1]

Loudon (17, p. 691) described it as a native of North America; introduced into Europe in 1818, and, while it produces blossoms there in great abundance, yet he is not aware of any producing ripened fruits. In America, according to Pursh (22, p. 332), they are succeeded by fruit, of the size of a pigeon's egg, dark purple and very good to eat.

Until 1825, *Prunus maritima* was described under various synonyms. Beck (2, p. 95) points out the similarity of descriptions among certain species, yet suggests that they may be distinct. Torrey (29, p. 194-5) and Torrey and Gray (30, p. 408) confirm Beck's suggestion and give a list of some of the synonyms used.

Prunus cerasifera	1789	Ehrhart	(12, p. 17)
Prunus acuminata	1803	Michaux	(20, p. 284)
Prunus sphaerocarpa	1803	Michaux	(20, p. 284)
Prunus pygmaea	1809	Willdenow	(34, p. 519)
Prunus pubescens	1814	Pursh	(22, p. 332)
Prunus littoralis	1824	Bigelow	(3, p. 193)

All these are now grouped, however, into the one species—now under consideration.

---

[1] Since the above was written, Wight (33, page 55) in a recent publication, dated April 2, 1915, arrived at the same conclusion.

Until 1904 all the work was taxonomic and consisted of descriptions more or less complete. At this time, Macfarlane (18, p. 216), pointed out marked variations in the fruits of the species under the following heads:—

(1) color
(2) weight
(3) size and shape
(4) consistence
(5) taste
(6) time of maturation
(7) comparison of the stones

all of which the writer has confirmed by independent observation and research eleven years later.

Bigelow (3, p. 193) had, however, previously (1824) called attention to three varieties, namely:—

Variety a. Fruit an inch in diameter, purple, with a glaucous bloom.
b. Fruit similar, but smaller.
c. Fruit crimson shining.

He further states, "This is our Common Beach Plum, much prized for its agreeable fruit, and deserving attempts at cultivation. I do not find it described by any author, unless possibly by Michaux under the name of *P. sphaerocarpa*, a name previously appropriated by Swartz to a West Indian species. From *P. maritima* of Pursh it appears widely different in its inflorescence, acumination, and fruit."

Torrey and Gray (30, p. 408) fourteen years later (1838) described two varieties.

Var. 1. Leaves softly pubescent or pubescent beneath; fruit large, pleasant. Corresponds to *P. sphaerocarpa*, Michaux; and *P. littoralis*, Bigelow.

Var. 2. Leaves when old mostly glabrous on both sides; fruit smaller, red or purplish. Corresponds to *P. pygmaea*, Willdenow and *P. acuminata*, Michaux. They state that these two forms may be traced into each other with great certainty; and Bigelow seems to have included both under his *P. littoralis*.

Stone (27) gives the best description of the distribution of the species in the state of New Jersey. On page 250 the writer gives a detailed technical description of *P. maritima*.

3. *Material and Methods*

The material for this study was first collected by the writer in early autumn of 1912, during the period of maturation of the fruit. Those bushes were tagged which showed marked variations in the fruits, and material was taken from them from time to time during the summer of 1913, in order to trace variations in the stem, bud, blossom, leaf, young fruit and mature fruit. As a check to my work, during the late summer and autumn of 1913, I tagged other bushes and collected material from them, as indicated above, during the summer of 1914. In this way, I worked in duplicate the second summer, and checked it with my work of the previous year. This should serve to strengthen my conclusions, as my observations were not taken from any one bush that I might arbitrarily select for my type, but rather from two to five tagged bushes. (By so doing, there was reasonable safety that the tags would not be torn off the bushes by the wind, or the rain.) Great difficulty was experienced in obtaining fully matured fruits from the bushes, since the natives gathered them just as they began to color and used them for making preserves. Alcohol of 30% strength was used as a killing fluid, and 70% was used as a preserving fluid. The material from Long Island and from Nantucket was collected by Dr. Harshberger during August 1913. As this material included only the leaf, stem and immature fruit, no attempt was made to study it as was done in the case of the New Jersey material.

4. *Distribution*

(1) Along the Coast—North and South

While this plant is a native of North America, and found only along the Atlantic coast, yet authors do not seem to agree upon its wide distribution. In Gray's New Manual of Botany (16, p. 498) the range is given as extending from Maine to Virginia. Britton (4, p. 524-5) states that it has been found growing on the sandy coasts of New Brunswick. Hutchinson (7) in an article in the Botanical Magazine (December 1909) confirms Britton's statement. As to its southern extension, Chapman (6, p. 131) records it from Alabama; the basis of this record is, however, a very imperfect specimen collected by Buckley in the Alleghany Mountains, and Sargent in his "Silva of North America" is probably justified in concluding that as no other trace of *Prunus maritima* has been met with in this now well explored region, the specimen obtained by Buckley merely represents a form of *P. Alleghaniensis* (Porter).

Sargent states (Silva of North America, 4(1892), p.28):—"There is preserved in the Herbarium of Columbia College a specimen of a Prunus collected in Alabama many years ago by Mr. S. B. Buckley, and referred by Torrey and Gray (30 v. 1.408) to their variety 2. of *Prunus maritima*, and in the same collection a specimen of what is described as 'a small tree 10-15 feet high; fruit oval, small, blue, glaucous, very austere to the taste,' and which was seen many years ago in Lincoln County, N.C. by Mr. M. A. Curtis, who mentions it in his report of the trees of that state (Rep. Geol. Sur. N. C. 1860, 3, 56). It is possible, as Professor Britton is inclined to believe, that these specimens represent a southern form of *Prunus Alleghaniensis*; but they are without flowers, and hardly suffice to justify the extension of the range of the species of which no other trace has been found in the now well explored region of the southern Alleghany Mountains."

In a catalogue of the plants of Louisiana published in 1849, *Prunus maritima* is given as growing there, but Professor R. S. Cocks, of Tulane University, who has made a study of the vegetation of that state, and, together with Professor C. S. Sargent, of the Arnold Arboretum, has collected plums in all parts of it, writes that they have not come across any specimen of *Prunus maritima*. While it may occur there, yet he has no good evidence that it does.

My interest in writing to Professor Cocks came from a specimen I saw preserved in the Herbarium of the Academy of Natural Sciences of Philadelphia, and collected by Dr. Lincecum about 50 years ago, along the sandy shores of Texas. He called it the Post Oak Plum. On the same sheet was a brief description from which I quote:—"Small shrub 1-2 feet high, two varieties, found in patches on the poorest sandy post ridges." I presume he means on the sand dunes which are covered with scattered growths of the Post Oak. As that habitat is very much like the one in which I collected my specimens, and the few characters which he gives seem to correspond with *Prunus maritima* of New Jersey, it leads me to believe that it was a closely related species of *Prunus*, modified by the different climatic conditions under which it grew. The specimen is in poor condition containing very few leaves or flowers. He further states:—"It blooms before the leaves and so numerous are they that the patches of the shrub are perfectly white and may be seen a great way. Fruit small and sour. Purple in color. Blooms in February."

The specimens and material, which I have seen and examined and from which the results of this paper were derived, range from Cape

Cod, Mass. to Lincoln County, N. C. The collections include the following places:—Falmouth and Nantucket, Mass., Oyster Bay, Long Island, and in New Jersey, at Island Heights Junction, Sea Side Park, Barnegat, Manasquan, Manahawkin, Brigantine Beach, Pleasantville, Ocean City, North Wildwood, Manumuskin, Maurice River and Cape May Point. The latter place was the most favorable for collection and study, as all the different varieties grow there, thus enabling me to compare them all in a single day.

Just across the bay from Cape May in Delaware, *Prunus maritima* var. *pygmaea* is reported; specimens of which can be seen at the Academy of Natural Sciences, Philadelphia, and which were collected at Rehoboth and Lewes, Delaware. It is characterized by smaller and more narrow leaves.

Prunus pygmaea, a specimen of which can also be seen at the Academy, and which resembles *Prunus maritima* var. *pygmaea* from Delaware, has been collected in Lincoln County, N. C. The specimen at the Academy is very old and shows only a few leaves.

(2) Inland

About forty miles inland and running almost parallel with the present shore line of New Jersey, beginning at a point near Salem, and extending in a northeasterly direction to near Brown's Mills, occur local isolated patches of *Prunus maritima*. Along this line, I have collected material at Hainesport, Medford, Clementon and Atco. While I have not collected any material, nor on my numerous excursions to the shore have I seen it growing in the Pine Barren region, yet it is reported by others, e.g. Stone (27), as growing there. This distribution is quite unusual as it is the only region, as far as I have been able to learn, where it is found growing so far from the sea. The question naturally arises why this distribution.

It has been suggested that birds or Indians may have carried them there. But the distribution of plants is now generally regarded as being connected with climatic or ecologic factors, it occurred to me therefore that a geologic explanation or suggestion at least was possible. The soil at Clementon and at Atco, where these plants grow, is composed of a light colored loose sand resembling in every respect our present beach sand. The rounded surfaces of the grains indicate that it is water worn. Occasionally the soil may be firm where strata of clay approach and form the surface. Most geologists regard this formation as Miocene. A few miles to the west is found the green sand marl or glauconite of the Upper Cretaceous. The marked character in the color of the sand easily separates the two formations. At a period in the early Cenozoic, when the waters of the Atlantic washed the land at a point near Clementon, *Prunus maritima* was evidently growing along the then sandy shore line, but as the ocean slowly retreated eastward and time passed, isolated patches of it seem to have become stranded. Further evidence of this is illustrated by Stone (27) in his distribution of *Prunus maritima* over the Pine Barren region to the east, which is geologically younger. With the on-coming of the ice, and the approach of the glacial period, we find that although this area was not glaciated yet the effects of the period upon the plant life could not have been more than to vary the type, if it were permitted to live at all, and that is just exactly what I have found, a variety with characters markedly different from any found along the coast. Only one variety, the small blue, is found there, which I have named—*P. maritima* var. *praecox*.

5. *Habitat and Environment*

Prunus maritima is found growing most abundantly on the drifting sand dune area along the Atlantic coast. At Cape May Point, where I have made extensive studies of the plant, I have found it growing within twenty-five yards of the limit of high tide. The thicket formation there is rather narrow, as back of the limited sand dune area is an extended marsh. To the south west, however, it extends for a distance of several miles, covering an area of many acres. At other places along the coast, the areas are much smaller, consisting in some places of only a few hundred bushes, while at Hainesport and at Atco we find isolated colonies of 50 to 100 bushes. The plants of this habitat consist of *Quercus falcata*, *Juniperus virginiana*, *Myrica carolinensis*, *Ilex opaca*, *Pinus rigida* *Rhus radicans*, *Sassafras officinale*, *Solidago sempervirens*, *Ammophila arenaria*, *Lathyrus maritimus*, *Hudsonia tomentosa*, etc. Regarding the inland distribution of the species it is quite interesting to note that the blossoms appear about two weeks earlier than those of the shore varieties, and the fruits are fully matured before the ones at the shore have begun to ripen. This is undoubtedly due to the environmental conditions under which they are growing, as it is true of many other species of shore plants, that they are often retarded in their development and growth in the spring by cold breezes from the ocean. Again I have noted that the blossoms on the bushes nearest the ocean are the last to open. Likewise, the smallest bushes bloom the earliest, and the lowest blossoms open the earliest, i.e., the blossoms at the tip of the branches are the last to open. The explanation obviously is the radiation of heat from the sun-heated sand.

II. STRUCTURE

1. *Stem*

(a) *External Structure*:—The Beach Plum is a bush which usually grows to a height of two to six feet and may then be from ten to twenty-five years old. Occasionally plants from twelve to fifteen feet high are found which indicate an age of eighty to one hundred years; the main stem or trunk then measuring three to three and one-half inches in diameter, at the level of the ground. The bark of the old stems is rough, fissured, dark brown to gray, and usually covered with a persistent growth of lichens. The younger branches are dark purplish gray with numerous irregular, flattened lenticels. On the youngest twigs the lenticels are more circular in outline and elevated. These twigs are usually of a light silvery gray color, due to an epidermis which is shed rather irregularly in the different varieties. After the shedding of the epidermis, which is usually completed by the beginning of the second year, the twigs are of a reddish brown color, the light gray lenticels being quite conspicuous. The epidermis, during the first year's growth, is slightly puberulous. The leaf scars are small and crescent shaped, and contain three small fibro-vascular bundle scars, which may be seen only by aid of a hand lens.

Branching:—The method of branching is quite marked and varies more with the situation in which the bush may be growing than with the variety. As far as I have been able to ascertain, the branching should not be considered a constant factor, although it may apparently seem to be from some of the plates incorporated in this thesis. The bushes exposed to the cold sea breezes, and the sand laden winds are the most stunted in their growth, the yearly additions being rarely more than two or three inches. As a result, the stems are more sturdy and more branched, like that shown on Plate LXVI, Fig. 1. This is quite typical of the large blue variety, and was taken from a bush bearing that color of fruit. The yellow variety Plate LXVI, Fig. 4 illustrates the other extreme of the series of photographs taken. This branch was taken from a bush growing five-hundred yards from the shore where the more sheltered conditions favored an elongated less branched type. However, when the yellow variety grows nearer the ocean it more closely resembles one of the red variety of which Plate LXVI, Fig. 3, is very typical. The purple variety, a specimen of which is shown on Plate LXVI, Fig. 2, shows spine like lateral branches, which although they are only shown as belonging to this variety, should not be considered as wholly characteristic of it. They may occur on the reds and blues, even though they are not shown on the photographs, but I have never found any on the yellows. This purple type grew under intermediate conditions as the branching would seem to indicate. The short lateral branches —which usually fruit well—shown on Plate LXVI, Fig. 1 will later become sub-spinescent and resemble the spine like branches of Plate LXVI, Fig. 2.

(b) *Internal Structure.* (microscopic):—The internal structure of the stem which is shown on Plate LXVIII in cross section conforms to previously described woody stems of the Rosaceae, of which the following parts may be summarized briefly:—*Epidermis* rather thin, dark reddish brown, which by the end of the first year is beginning to be shed. By the end of the third or fourth year a thin bark has formed which sloughs off in thin persistent scales or plates. Beneath is a layer of *cork* from fifteen to thirty cells thick, which gradually pushes outward to form the bark as new cork cells are cut off. These protective coverings surround a rather thin *cortex* composed of ten to fifteen rows of parenchyma cells; included in which are numerous clustered crystals of calcium oxalate and numerous irregular masses of sclerenchymatous tissue or stone cells. The latter are distributed around the stem in the inner portion of the cortex. Beneath is a ring of rather loosely arranged *phloem* tissue which extends outward from the wood in curious finger like projections. Numerous air spaces are thus formed between them and the rows of pith cells as they extend outward to join the cells of the medullary rays with those of the cortex. The *wood*, which is composed of narrow annular growths, is hard and compact. It contains numerous water conducting vessels which vary in relative number in the different varieties. It is also characterized by many broad medullary rays, widened-out extensions of which separate the phloem into the finger like projections mentioned before as they pass outward to join the cortex cells. The *pith*, which comprises the central portion of the stem, is solid and compact, while the cells of it store starch during the resting period. Clustered crystals of calcium oxalate are frequently but irregularly distributed throughout it.

2. *Leaf*

(a) *External Structure*:—The leaves opening with or after the blossoms, rarely before, vary in shape from oval to ovate to obovate. They are rather firm in texture, greenish above paler beneath, and more pubescent when young. This pubescence is due to hyaline, unicellular, epidermal hairs. The stipules are linear, lobed, pubescent, glandular-serrate and very early deciduous. The leaves are more or less rounded at the base, acute at the apex, finely and sharply serrate with glandular tipped teeth when young. At the junction of the lamina and petiole are two rather large conspicuous glands. In regard to leaf variation, in lamina and petiole considerable diversity exists, and the main types that are associated with the varying colors of fruits are set forth in the subjoined table.

VARIETY	SIZE OF LEAF	LENGTH OF PETIOLE
Large Blue	3.5 x 6.8 cms.	1.0 cms.
Small Blue (1)	3.0 x 5.7 "	1.0 "
(2)	2.5 x 5.5 "	.7 "
Large Purple	3.5 x 6.8 "	1.2 "
Small Purple	2.0 x 4.0 "	1.0 "
Large Red	3.2 x 6.8 "	1.1 "
Small Red	2.0 x 4.5 "	.9 "
Large Yellow	3.5 x 6.8 "	.8 "
Small Yellow	2.5 x 5.7 "	1.0 "

The above measurements are the average of ten selected leaves. Small Blue (1) is var. *coerulea parva* from Cape May Point; (2) var. *praecox* from Hainesport, N. J. The leaves are puberulous on the upper surface along the midrib in all varieties, while in the blues and in the yellows the pubescence seems to exist along the main veins as well. In the reds and in the purples the upper surface of the blade appears glabrous except along the midrib. On the lower epidermis the pubescence is located along the midrib and in the axils of the main veins. The small inland blue var. *praecox* appears as pubescent on the lower surface as on the upper, while the small yellow var. *lutea parva* is pubescent along both veins and cross veins, but not as pubescent on the lower as on the upper surface. These details are set forth in comparative order in the table, on the following page.

Small Blue (1) var. *coerulea parva*; (2) var. *praecox*.

The pubescence of the small yellow is more dense on the upper epidermis, and appears to be very characteristic of it. Stomata occur only on the lower surface in island-like formations. Sometimes these islands are irregular and indistinct, i.e., broken up into a number of smaller ones, which makes it rather difficult to get an accurate account of the number of stomata in an island area. The midrib, veins and nerves

VARIETY	UPPER EPIDERMIS	LOWER EPIDERMIS
Large Blue	Pubescent along mid rib and leading veins	Pubescent only along midrib
Small Blue (1)	Pubescent only along midrib	Pubescent only along midrib
(2)	Pubescent along midrib, veins and cross veins	Very pubescent as described for up. epid.
Large Purple	Pubescent only along midrib	Pubescent only along midrib
Small Purple	Pubescent only along midrib	Pubescent only along midrib
Large Red	Pubescent only along midrib	Pubescent only along midrib
Small Red	Pubescent only along midrib	Pubescent only along midrib
Large Yellow	Pubescent along midrib slightly along the leading veins	Pubescent only along midrib
Small Yellow	Pubescent over whole surface, midrib, veins and cross veins	Pubescent along midrib, veins and cross veins

are sharply prominent beneath, where 6 to 8 veins usually occur on each side of the midrib.

(b) *Internal Structure* (microscopic). The leaf is characterized by a thick epidermis that prevents too rapid transpiration of water. The cells of the upper epidermis are 2 to 2 ½ times larger and more dense than those of the lower. Frequently mucilage is deposited in the cells of the upper, less often in the cells of the lower. Two rows of palisade cells can usually be distinguished, but these are sometimes small and hard to differentiate. The mesophyll is composed of an undifferentiated mass of minute cells, which is very characteristic of all varieties, the air spaces are numerous, but small, while along the midrib and main veins are numerous clustered crystals of calcium oxalate. These vary in size and relative number in the different varieties. In cross section, the crystals are seen to be scattered throughout the mesophyll, sometimes in the palisade layers near the upper epidermis, at other times in the undifferentiated mesophyll near the lower epidermis, but never so far as I have been able to discover, are they located in the epidermis. Frequently, these crystals vary in size in the same leaf and may be classified as large, medium, and small. The midrib contains a deeply crescentic vascular bundle that occupies one-half of its area and is surrounded by a loose cortex of mesophyll cells, which, like the leaf mesophyll, contains crystals. The crescentic shaped patch of xylem consists of a mass of woody or lignified tissue. Its elements are arranged as a radiating series of cells. These are surrounded on the lower side by a crescentic patch of phloem in the outer zone of which are numerous mucilage cells and crystals of calcium oxalate.

3. *Flower*

The flowers of *P. maritima* are white, with a pinkish tinge in the blue and in the purple varieties. This is especially noticeable in the sepals and stamens after the petals have fallen, although some of the purple variety have decidedly pinkish petals. They are borne in umbels with 2 to 5 flowers (usually 3) in a cluster near the ends of the youngest twigs, or on the short lateral spinescent branches of the older wood. The period of blooming lasts from ten days to two weeks. It ranges from April 19th, for the small inland blue (observation made at Hainesport, N. J.) to May 15th for the late varieties at Cape May Point. In most of the varieties the blossoms appear before the leaves, yet in some of the more protected bushes the leaves are well developed before the blossoms fall. Evidence of this may be seen on some of the accompanying plates. The writer has no accurate date as to the periods in other places north and south, but their relative periods of blooming can easily be approximated from the time stated for the New Jersey varieties. The pedicels are very pubescent; the hairs on the reds and purples being the longest, while those on the blues and yellows are relatively short. The sepals are green with pinkish margins as noted above, concave, pubescent, uniting to form a campanulate receptacular tube 2 to 3 mm. deep. The deepest cups are found on the varieties bearing the larger sized fruits. The sepals vary in length from 2 to 4 mm. and from 1 to 2 mm. in width. The outer surface of the receptacular tube is very pubescent, while the inner concave surface is completely overspread by an orange yellow glandular tissue which secretes the abundant nectar. The petals are white with a slightly pinkish tinge in the blue and purple varieties, rounded at the apex and contracted at the base into claws. They vary in width from 4 to 7 mm. and in length from 6 to 10 mm. The corolla spreads out to form a relatively flat surface of which the diameter is 15 to 20 mm. in the large and 10 to 15 mm. in the small varieties. The stamens are variable in number, but usually about 30 of which some are frequently abortive; 25 being the average number in the small inland blue, 35 in the large blue black. These vary in length from 1-2 mm. in the small abortive ones nearest the center, to 5-10 mm. in the outermost ones, and are inserted on the edge of the receptacular tube. The anthers are two lobed and bear numerous triangular-shaped pollen grains. The flowers are apparently proterogynous, since the stigma matures before the flower is fully open and may easily be seen protruding between the petals of a partly opened flower bud, but homogamy is probably not uncommon. The style projects in the middle of the flower beyond the obliquely diverging stamens, and is 10 to 20 mm. long. The ovary is one celled and characterized by two pendulous ovules. Frequently the flowers are andro-dioecious which would account for the profusely abundant blooms matured on every bush during April and May, and also for the total lack of fruits on some bushes, the rarity on others, and the extreme abundance on still others. The last noted would naturally be those that developed stamens and a well formed pistil with swollen ovary and elongated style. At Hainesport, N. J. the writer has observed bushes for three years, during which time they have never set fruit, yet have constantly bloomed in great profusion. The same condition prevails in other localities. The flowers are insect pollinated as is indicated by the vast horde of Hymenoptera which frequent them during the warm part of the day. The showy white corolla and the nectary are the chief attractions for insects as the flowers are practically odorless. Cross pollination is favored by most writers, and such a view seems likely from the fact that the bee in order to obtain the nectar must get far into the interior of the flower. In so doing it seems hardly possible for it to miss touching the stigma.

Knuth states that automatic self-pollination appears regularly to take place in hermaphroditic flowers of the *Pruneae* should insect visits fail. Whether this is effective or not seems doubtful, as Kirchner states that numerous bushes observed by him rarely set fruit. The true explanation, however, seems to be found in the andro-dioecism as described above by the writer.

4. *Fruit*

The fruits exhibit by far the most striking variation to the eye, and it was this variation which caused Dr. Macfarlane (18) to first make a preliminary study of the Beach Plum. As I have inferred under materials and method, I began making observations and collecting material when the bushes were in fruit as the variation at that time was the most easily recognized. The fruit, which is usually produced in large quantities, is borne on the younger branches mostly of the previous year's growth. They begin to ripen about the first of August in the earlier varieties and continue to produce an abundance of fruit until the latter part of September when the best varieties have fallen. There is, however, a variety of the small purple, which does not mature all its fruits until frost overtakes it early in October.

It is interesting to note that the fruits remain upon the bushes about three weeks after they have ripened, a much longer time than we find among our cultivated varieties. Likewise, it may be of interest to know that one rarely finds any of the fruits rotting on the bushes; and after they have fallen, the fruits, if not carried away by birds, or other animals that distribute fleshy fruits, are palatable for another week, after which the pulp cells begin to disintegrate, but the leathery skin prevents them from rotting until they finally dry into a shrunken shriveled mass. The fruit is sub-globose or slightly oval. The skin is tough and leathery, of a blue, purple, red or yellow color, covered with a light waxen bloom, and flecked with numerous small light-colored spots. The flesh is of a watery yellow color, juicy, astringent and in most varieties decidedly free from the stone. The stone is rounded or flattened, pointed at both ends, rigid on the ventral, and slightly grooved on the dorsal suture. The taste depends largely upon the presence or absence of the tannin content. Sugar is evidently distributed throughout the pulp, while tannin is deposited in a layer just beneath the skin. Mucilage is very abundant, and is contained in ducts or canals imbedded in the pulp. The yellow fruits seem to be rather free from the tannin and consequently do not have a sharp, astringent taste. That they vary in size is shown from the following table.

VARIETY	DIMENSIONS	LENGTH OF STEM
Large Blue	1.7 x 1.6 cms.	1.4 cms.
Small Blue (1)	1.5 x 1.4 "	1.9 "
(2)	1.2 x 1.3 "	1.1 "
Large Purple	1.7 x 1.6 "	1.4 "
Small Purple (1)	1.3 x 1.2 "	1.7 "
(2)	.9 x 1.0 "	1.4 "
Large Red	1.6 x 1.5 "	1.5 "
Small Red	1.4 x 1.3 "	1.2 "
Large Yellow	1.7 x 1.6 "	1.2 "
Small Yellow	1.4 x 1.3 "	1.5 "

The length of the stem, as shown above, is quite variable, and while it is not constant and does not seem to explain much, yet it serves to show the great variation which this plant exhibits. Small Purple (2) noted in the table above illustrates the variety which does not ripen until October.

Color. The red, purple, and blue colors seem to be due to one or more pigments added to the yellow color. This became apparent when I added a dilute solution of NaOH to the skin of the red variety. The red dissolved pigment was readily attacked by the alkali and quickly changed its color to purple, to green and finally to yellow. Small yellow chromatophores were easily distinguished after the disappearance of the dissolved pigment, and the pulp, which was treated in a similar manner, though containing only a small amount of pigment, had the same general appearance as the yellow variety. This seems to prove, as far as color goes, that the yellow is the more primitive type and that the red has taken on an added pigment. In the purple and in the blue varieties there appears a sub-epidermal layer which in addition to the red dissolved pigment (same as in the red varieties) gives to the fruits their respective colors. This pigment is contained in small oval or rod shaped bodies which are considerably smaller than the cells which contain the red dissolved pigment. The skin of the purple and of the blue varieties resembles the skin of the red after the inner pigmented layer has been removed. This further emphasizes the fact that the yellow is the more primitive type, since the purples and the blues have evolved from the reds by the increasing alkalinity of a dissolved red pigment, and again the reds have evolved from the yellows by the formation of a dissolved acid pigment, and the disappearance of the yellow color from the chromatophore. To say that these varieties overlap, and that the colors grade into each other i.e., that the purples grade into the reds on one side, and into the blues on the other, may appear to weaken the value of the varietal characters given later. But when one considers the sum total of varietal characters typical of each fruiting type, the distinctions above indicated for the fruit seem to hold fairly well for other parts, and to constitute sufficiently stable diagnostic differences.

The majority of the fruits in any one locality are of the dark blue variety, and comprise about 75% of all the plants. The yellow is the least common and forms about 1%, the reds about 5% and the purples not over 20%. The only locality where I have seen the yellow variety growing was at Cape May Point, although it has been reported as growing at Island Heights Junction, N. J., and at Falmouth, Mass. The other varieties are quite common and can be found in almost any locality where the Beach Plum grows.

The earliest ones to ripen are the small blue var. *praecox*, which are fully matured by August first. These, as I have noted earlier in this paper, blossom about ten days earlier than any of the other varieties.

The next to ripen, and the first at Cape May Point, are the large blues which are fully matured by August fifth, and continue until about August twenty-fifth. They ripen gradually on the bush like our cultivated plum, and there may be found on any of the bushes fruits in all degrees of maturity. The yellows, however, differ from the others in this respect, as the fruits appear to all mature about the same time. As the blues ripen, they first turn pinkish on one side and this gradually deepens into a deep red. At this stage, they closely resemble the large red variety, and could easily be mistaken for them if they matured at the same time, but in about four days they will have turned to a dark blue and are now covered with a light waxen bloom. About the time that the blues are fully matured, the yellows begin to produce abundant fruits, which are characterized by their sweet, pleasant taste. The large purple variety ripens next, followed shortly by the red. Finally, about the last of September, the small purple ripens, the coarsest and smallest fruited variety. They are by far the richest in tannin and decidedly unpleasant to the taste. Usually frost overtakes them before they attain what may be termed ripeness.

VARIETY	PERIOD OF MATURATION
Large Blue	Aug. 5—Aug. 25
Small Blue (1)	Aug. 10—Aug. 30
(2)	Aug. 1—Aug. 20
Large Purple	Aug. 15—Sept. 5
Small Purple	Sept. 1—Oct. 1
Large Red	Aug. 25—Sept. 15
Small Red	Aug. 25—Sept. 15
Large Yellow	Aug. 10—Aug. 30
Small Yellow	Aug. 10—Aug. 30

The fruit is subject to the sting of an insect, the plum weevil or plum curculio as it is sometimes called. The stinging occurs either when the blossom is on or shortly after it has fallen. The very young fruits can then readily be detected when punctured by the insect, owing to a mass of mucilage exuding from the wound. The blues and the purples are the most generally attacked, and often more than half of the fruits are stung. The reds are occasionally attacked while the yellows are seldom, if ever, stung. Contrary to expectation, only a few of the stung fruits fall to the ground, while the majority of them remain upon the bush even to maturity. There results from the sting a hard black core of hardened mucilage, which becomes a localized center for the secretion of tannin, and this gives to the fruit a most unpleasant taste.

During the early stages of growth, the young fruit is subject to a fungus attack by *Exoascus Pruni* (Plum pocket). This is the same fungus that attacks our cultivated plum and which has been fully described by De Bary and Duggar.

Fruit Weight:—It is possible under this head to give statistics which will emphasize genetic peculiarities and with which I was able to correlate varietal characters in the leaf and flowers.

VARIETY	WEIGHT OF FRUIT	WEIGHT OF STONE	WEIGHT OF FLESH	PER CENT OF FLESH
Large Blue	3.61 grams	.54 grams	3.17 grams	88
Small Blue (1)	1.74 "	.22 "	1.52 "	87
(2)	1.29 "	.18 "	1.11 "	86
Large Purple	3.52 "	.43 "	3.09 "	86.5
Small Purple (1)	1.40 "	.22 "	1.18 "	84
(2)	.55 "	.18 "	.37 "	67
Large Red	2.82 "	.40 "	2.42 "	86
Small Red	1.71 "	.28 "	1.43 "	84
Large Yellow	2.92 "	.40 "	2.52 "	86
Small Yellow	1.70 "	.22 "	1.48 "	87

These variations in weight are very constant for each variety. The weights were obtained by weighing 10 plums and then estimating the average. The per cent of flesh ranges from 84 to 88 per cent with the exception of the small purple late variety which does not ripen until October. The table shows that the stone has attained nearly its full size and weight while the flesh development is quite scanty, owing to the lateness of its maturity.

Stone Variation:—By way of comparison the stone variation is given and seems to markedly correspond with the variation as shown above in the size and weight of the fruits.

VARIETY	LENGTH	THICKNESS
Large Blue	12.5 m.m.	11.0 x 7.3 m.m.
Small Blue (1)	11.0 "	7.5 x 5.8 "
(2)	7.8 "	6.5 x 5.4 "
Large Purple	13.5 "	10.0 x 6.5 "
Small Purple	8.5 "	7.8 x 5.5 "
Large Red	12.5 "	9.5 x 6.5 "
Small Red	9.0 "	8.5 x 5.8 "
Large Yellow	10.0 "	9.5 x 7.5 "
Small Yellow	8.5 "	7.0 x 6.0 "

Cell Contents and Glandular Structures

1. *Calcium oxalate* is excreted into cells of this plant as a waste product in considerable abundance. The evidence of it is shown by the rosette aggregate of crystals found in different parts of the plant. They are of various sizes and usually occupy an entire cell. The greatest abundance of them is found in the cortex of the stem, and in the loose parenchyma cells of the leaf mesophyll, where they are irregularly scattered throughout. Likewise, they are found in the sepals and receptacular tube. Again in the fruit, they are found not only in the soft pulpy cells and in the cells which are thickening their walls to form the stone, but also in the developing embryo inside. That they may be found in other parts of the plants is, in the opinion of the writer, very probable, as no special attempt was made to study them, their presence simply being noted in the histological study of the above named parts.

2. *Mucilage* is likewise excreted in great abundance, evidence of which is found in the cells of the upper and of the lower epidermis of the leaf. Mucilage canals are also found in the fleshy part of the fruit, and when the fruits are stung by the plum weevil masses of the mucilage exude from the puncture which soon hardens into a plug healing up the wound. When the ripe fruits are plucked from the stalks Macfarlane (18, p. 226) refers to the considerable amount of "bleeding" which occurs when they are heaped in a basket or on a dish for a few hours. This secretion, which is sticky to the touch, is evidently the same mucilage as that previously referred to. If the stalk is plucked with the fruits this bleeding is entirely prevented. Frequently when a branch is broken from the bush or an injury to the bark occurs, mucilage is exuded to cover the exposed wound.

3. *Tannin* which gives to the fruits their bitter astringent taste is deposited in a layer beneath the skin. The yellow varieties are almost free from it, while the late maturing ones contain the most; the small purple being extremely bitter. The intermediate types vary in tannin content. The softer and larger varieties when ripe contain only small traces of tannin.

4. *Glands* of the most interesting character are found on various parts of the plant, viz., at the junction of the lamina with the petiole, on the lobes of the deciduous stipules and on the teeth of the lamina. These are all fairly large—visible to the unaided eye—of a dark brown color, and very dense. The glands at the junction of the lamina with the petiole are borne on short stalks. Normally, there are two—one on each side of the petiole—but frequently one of these may be absent, while very rarely leaves are found with no glands developed. The

glands on the stipules are very conspicuous, but probably rank second in importance as the stipules are very early deciduous and consequently the glands are seen only for a short time. A reproduction of them is shown in Plate LXVIII, Fig. 11. The glands on the teeth of the lamina are more numerous near the apex of the leaf, and seem to be more common on the young leaves. Many of the older leaves do not show them.

III. Classification of Varieties

In dealing with this phase of the subject, I will give a detailed taxonomic description of the species as a whole after the style of Sargent. Such a description has not been attempted so far as the present literature goes and for that reason is needed all the more. Then I will give a detailed description of each variety as I have found them. To have established new species on the basis of the variation presented seemed to me rather to confuse, and for that reason I have decided to describe them as varieties. I might add that some of my varietal characters are equally as strong as the characters which were used in separating *P. Gravesii*, which grows in the immediate neighborhood and under precisely the same conditions as *P. maritima*.

Prunus maritima, Marsh.
(Beach Plum)

A small slender shrub, occasionally 12 to 15 feet high, with a main stem which is sometimes 3 1/2 inches in diameter, and which divides into numerous erect rigid branches; usually 2 to 5 feet high, erect or prostrate, forming dense thicket like clumps. *The bark* of the trunk is dark brown to gray, 1/8 to 1/4 inches thick, the fissured surface being broken into thin persistent scales. The *branches* are stout, rigid, marked with minute pale lenticels, puberulous during the first summer, and unarmed with occasional lateral spinescent or spur-like branchlets. The *winter buds* are about 1/8 inch long, obtuse, and covered with chestnut brown scales. The *leaves* are oval, ovate or obovate, 4.0 to 6.8 cms. long by 2.0 to 3.5 cms. wide, on short, stout pubescent petioles, 7 to 12 mm. long; acute, finely and sharply serrate with glandular tipped teeth when young; at the base of the blade are two, occasionally one, rather large, round, stalked, dark glands, sometimes eglandular; slightly puberulous to glabrous on the upper surface, while on the lower surface quite pubescent along the midrib and in the axils of the primary veins, slightly along the cross veins of certain varieties. Stipules linear, lobed, pubescent, glandular serrate and very early deciduous.

The flowers, which appear from the middle of April to the middle of May before or with the unfolding of the leaves, are borne in 2 to 4 flowered umbels, 10 to 15 mm. broad when fully expanded; pedicels and calyx rather strongly pubescent, pedicels 7 to 15 mm. long; calyx tube campanulate 2 to 4 mm. deep, calyx lobes ovate, rounded at the apex with inflexed edges, pubescent on both sides. Petals white or pinkish, oblong or oblong ovate, 7 mm. long and 4 mm. broad, contracted to claws at the base. Stamens 25 to 35, filaments of two lengths; style stout, 10 to 15 mm. long, glabrous and truncate at the tip. Carpel ellipsoid, glabrous containing two pendulous ovules. *The fruit* which is frequently produced in great quantities, ripens from August 1 to October 1; it is borne on short stout stems, usually of the previous year's growth sub-globose to slightly oval, and varies in size from 1.0 to 1.7 cms. in diameter to 1.0 to 1.6 cms. long. The skin is thick and rather tough, blue, purple, red or yellow, covered with a light waxen bloom and abunddantly flecked; the flesh is of a watery greenish yellow, juicy astringent and decidedly free from the turgid stone which is rounded or compressed. The stone varies in size from 6.5 x 5.4 mm. to 11. 0x7. 3 mm in diameter and 7.8 to 12.5 mm. long, pointed at both ends, ridged on the ventral, slightly grooved on the dorsal suture.

1. *Prunus maritima var. coerulea magna*
(Large Blue Black)

Form	A low spreading bush 3 to 6 feet high forming dense thicket like patches, branching very irregular with short, stout branches which usually stand erect, giving it the aspect of gnarled and stunted in its habit of growth.
Bark	Old stem rather stout, rough, cracked into thin dark gray plates which tend to persist, mostly lichen covered. Young branches smooth, dark purplish brown, numerous light colored lenticels, and numerous spinescent lateral branches. Youngest twigs smooth, numerous, elongate and rather stout, puberulous, epidermis silvery gray and when shed uncovers a light reddish brown bark.
Lenticels	Numerous, small, conspicuous, irregularly distributed over the branches, rounded and more elevated on the youngest twigs, darker and flattened on the young branches.
Buds	Alternate, conical shaped, sharp pointed, brown to gray covered with numerous triangular scales, puberulous along the margins; covered with a silvery gray waxy coating which is shed in early spring.

	Flower Buds more precocious in early spring, chestnut brown, rather abundantly scattered along the twigs, frequently compounded with a single leaf bud. *Leaf Buds* smaller, more pointed, dark gray and terminal, bursting after the bush is in full bloom.
Leaf Scar	Crescent shaped, with three small inconspicuous fibro-vascular bundle scars.
Leaf	Ovate, alternate, 3.5 cms. wide 6.8 cms. long, petiole 1.0 cms. long and finely pubescent; acute finely and sharply serrate with small dark brown glandular tipped teeth; rounded and biglandular at the base with two dark brown stalked glands; slightly pubescent along the midrib, very slightly along the primary veins on the upper surface, pubescent along the midrib and in the axils of the primary veins on the lower surface; stipules lobed, glandular-serrate, and early deciduous. At maturity thick and firm, dark green above, paler below.
Flower	Appearing from May 1 to 15 slightly before the leaves, 15-20 mm. broad on pubescent pedicels 15 mm. in length, usually in 3 or 4 flowered umbels, sepals ovate, rounded at the apex, pinkish along the margins, pubescent; calyx tube campanulate 2 mm. deep, glandular on the inner surface, pubescent on the outer; petals white turning pinkish on fading, ovate, rounded at the apex, contracted at the base into short claws; stamens 35, from 8 to 12 mm. long, glabrous, turning pink on fading, inserted on the calyx tube; style 15 mm. long, glabrous with truncate stigma; carpels simple, ellipsoid, glabrous.
Fruit	Ripening August 5 to 25 of a deep blue black color, spherical or slightly flattened at the blossom end 17x16 mm. covered with a light waxen bloom, flecked with numerous light colored spots; weight 3.0 to 3.9 grams, flesh of watery green color; pulp free from the stone, sweet and pleasant, abundance of tannin in a layer beneath the skin; stone oval, compressed, reddish in color, 12.5 mm. long and 11.0x7.3 mm. thick, weight .54 gram, acutely ridged on the ventral, slightly grooved on the dorsal suture.

2. *Prunus maritima Var. coerulea parva*

(Small Blue Black)

Form	Low spreading bush 3 to 6 feet high, branching irregular, gnarled and stunted in its habit of growth.
Bark	Old stem rather slender, elongated, rough, cracked into thin dark gray plates which tend to persist. Young branches smooth dark purplish brown with numerous light gray flattened lenticels, and few spinescent branches. Youngest twigs smooth, numerous, elongate, slender, puberulous, epidermis silvery gray and when shed uncovers a light reddish brown bark.
Lenticels	Numerous, small, rather inconspicuous, irregularly distributed over the branches, rounded and elevated on the youngest twigs, darker and more flattened on the younger branches.
Buds	Alternate, conical shaped, sharp pointed, brown to gray covered with numerous triangular scales, puberulous along the margins; covered with a silvery gray waxy coating which is shed in early spring. *Flower buds* lateral, more precocious in early spring, chestnut brown, very abundantly scattered along the branches, frequently compounded with a single leaf bud. *Leaf buds* smaller, more pointed, dark gray and terminal bursting after the bush is in full bloom.
Leaf Scars	Crescent shaped with three small inconspicuous fibrovascular bundle scars.
Leaf	Ovate, alternate, 3.0 cms. wide 5.7 cms. long, petiole 1.0 cms. long and finely pubescent; acute, finely and sharply serrate with small dark brown glandular tipped teeth; rounded and biglandular at the base with two dark brown stalked glands; pubescent only along the midrib on the upper as well as the lower surface, blade glabrous; stipules lobed, glandular-serrate, and very early deciduous. Thick and firm at maturity, dark green above, paler below.
Flower	Appearing from May 1 to 15 slightly before the leaves, 14 to 18 mm. broad on pubescent pedicels 13 mm. in length, usually in 3 or 4 flowered umbels, sepals ovate, rounded at the apex, pinkish along the margins, calyx tube campanulate 2 mm. deep, glandular on the inner surface,

pubescent on the outer; petals white turning pinkish on fading, ovate, rounded at the apex, contracted at the base into short claws; stamens 35, from 8 to 10 mm. long, glabrous, turning pink on fading, inserted on the calyx tube; style 12 mm. long, glabrous with truncate stigma; carpels simple, ellipsoid; glabrous.

Fruit — Ripening August 10-30, of a deep blue color, sub-globose to slightly oval, 15 x 14 mm. covered with a light waxen bloom, flecked with numerous light colored spots; weight 1.4 to 1.85 grams; flesh of a watery green color, pulp free from the stone, sweet and pleasant, abundance, of tannin deposited in a layer beneath the skin; stone oval to elongate slightly compressed reddish in color, 11 mm. long and 7.5 x 5.8 mm. thick, weight .22 grams, acutely ridged on the ventral, slightly grooved on the dorsal suture.

3. *Prunus maritima Var. praecox*
(Small Inland Blue Black)

Form — A low spreading bush 3 to 5 feet high forming dense thicket-like patches, branching irregular with slender short branches, erect or prostrate often drooping forming a broad crown.

Bark — Old stem rather slender, rough, cracked into thin dark gray plates which tend to persist, mostly lichen covered. Young branches smooth, dark purplish brown, numerous light colored lenticels and numerous spinescent branches. Youngest twigs smooth, numerous, short and stout, puberulous, epidermis silvery gray and when shed uncovers a light reddish brown bark.

Lenticels — Numerous, small, conspicuous, irregularly distributed over the branches, rounded and elevated on the youngest twigs, while darker and flattened on the young branches.

Buds — Alternate, conical, sharp pointed, brown or gray, covered with numerous triangular scales, puberulous along the margins, covered with a silvery gray waxy coating which is shed in early spring.

Flower buds more precocious in early spring, chestnut brown, very abundantly scattered along the branches, frequently compounded with a single leaf bud near the end of the twigs.

	Leaf buds smaller, more pointed, dark gray, and terminal, bursting when the bush is in full bloom.
Leaf Scar	Crescent shaped, with three small inconspicuous fibrovascular bundle scars.
Leaf	Ovate, 5.5 cms. long and 2.3 cms. wide, petiole .7 cm. long, apex acute, finely and sharply serrate wtih small dark brown glandular teeth; rounded and biglandular at the base with two dark brown stalked glands; pubescent along midrib and primary veins above, very pubescent below along midrib, primary veins and cross veins; stipules lobes, glandular-serrate and early deciduous. At maturity thick and firm, dark green above, paler below.
Flower	Appearing from April 20 to May 5, slightly before the leaves, 10 to 15 mm. broad on pubescent pedicels, 10 mm. in length, in 2 to 4 flowered umbels, sepals ovate, rounded at the apex, pinkish along the margins, pubescent, calyx tube campanulate 2 mm. deep, glandular on the inner surface, pubescent on the outer, petals white turning pinkish on fading, ovate, rounded at the apex, contracted at the base into short claws; stamens 25, from 5 to 10 mm. long, glabrous turning pinkish on fading, inserted on the calyx tube; style 12 mm. long, glabrous with truncate stigma, carpels simple, ellipsoid, glabrous.
Fruit	Ripening August 1 to 20, of a deep blue black color, spherical or slightly flattened at the blossom end, 11.5 x 12.5 mm. covered with a light waxen bloom, flecked with numerous light colored spots; weight 1.1 to 1.6 grams, flesh of a watery green color, pulp free from the stone, sweet and pleasant, layer of tannin beneath the skin; stone oval to rounded, reddish in color, 7.8 mm. long and 6.5 x 5.4 mm. thick, weight .10 to .15 grams, ridged on the ventral, slightly grooved on the dorsal suture.

4. *Prunus maritima Var. purpurea magna*

(Large Purple)

Form	A low spreading bush 3 to 6 feet high, forming dense thicket like patches; branching rather regular and erect, with numerous spinescent lateral branches, general appearance less gnarled and stunted in its habit of growth.

Bark	Old stem rather stout, rough, cracked into thin dark gray plates which tend to persist, frequently lichen covered. Young branches smooth, dark purplish brown, numerous light colored lenticels, and numerous spinescent lateral branches. Youngest twigs numerous, elongate and stout, puberulous, epidermis silvery gray rather persistent, when shed uncovers a light reddish brown bark.
Lenticels	Numerous, large, very conspicuous, irregularly distributed over the branches, rounded and more elevated on the youngest twigs, darker and flattened on the young branches.
Buds	Alternate, conical shaped, sharp pointed, brown to gray covered with numerous triangular scales, puberulous along the margins; covered with a silvery gray waxy coating which is shed in early spring. *Flower buds* more precocious in early spring, chestnut brown, rather abundantly scattered along the twigs, frequently compounded with a single leaf bud near the end of the twigs. *Leaf buds* smaller, more pointed, dark gray and terminal, opening after the bush is in full bloom.
Leaf scar	Crescent shaped, with three small inconspicuous fibrovascular bundle scars.
Leaf	Ovate, alternate, 6.8 cms. long and 3.4 cms. wide; petiole 1.2 cms. long and finely pubescent; acute, finely and sharply serrate with small dark brown glandular teeth; rounded and biglandular at the base with two dark brown stalked glands; pubescent along the midrib on the upper surface, along the midrib and in the axils of the primary veins on the lower surface; stipules lobed, glandular-serrate, and early deciduous. At maturity thick and firm, dark green above, paler below.
Flower	Appearing from May 1 to 15 slightly before or with the leaves 15 to 20 mm. broad on pubescent pedicels 15 mm. in length, usually in 3 or 4 flowered umbels, sepals ovate, rounded at the apex, pinkish along the margins, pubescent; calyx tube campanulate 2 mm. deep, glandular on the inner surface, pubescent on the outer; petals white turning pinkish on fading, ovate, rounded at the apex, contracted at the base into short claws; stamens 30, from 8 to 12 mm. long, glabrous turning pink on fading, inserted on the calyx tube; style 20 mm. long glabrous with truncate stigma; carpels simple ellipsoid, glabrous.

Fruit	Ripening August 15 to September 5, of a reddish purple color, spherical to slightly oval 17 x 16 mm. covered with a light waxen bloom, flecked with numerous light colored spots; weight 3.25 to 3.6 grams; flesh of a watery greenish yellow color, pulp decidedly free from the stone, sweet and pleasant, tannin layer deposited beneath the skin; stone oval and compressed, reddish in color, 13.5 x 10.0 mm. and 6.5 mm. thick, weight .40 to .50 grams, acutely ridged on the ventral, slightly grooved on the dorsal suture.

5. *Prunus maritima Var. purpurea parva*

(Small Purple)

Form	A low spreading bush 3 to 6 feet high forming dense thicket like patches; branching rather regular, erect, with numerous spinescent lateral branches. General appearance less gnarled and stunted.
Bark	Old stem rather stout, rough, cracked into thin dark gray plates which tend to persist, mostly lichen covered. Young branches smooth, dark purplish brown, numerous light gray lenticels and numerous spine-like lateral branches. Youngest twigs smooth, numerous, elongate and rather stout, puberulous, epidermis silvery gray and when shed uncovers a light reddish brown bark.
Lenticels	Numerous, large, very conspicuous, irregularly distributed over the branches, rounded and more elevated on the youngest twigs, while darker and more flattened on the young branches.
Buds	Alternate, conical shaped, sharp pointed, brown or gray, covered with numerous triangular scales, puberulous along the margins; covered with a silvery gray waxy coating which is shed in early spring. *Flower buds* more precocious in early spring, chestnut brown, rather abundantly scattered along the twigs, frequently compounded with a single leaf bud near the end of the twigs. *Leaf buds* smaller, more pointed, dark gray and terminal, bursting after the bush is in full bloom.
Leaf Scar	Crescent shaped, with three small inconspicuous fibro-vascular bundle scars.

Leaf	Ovate to obovate, alternate 2.0 cms. wide 4.0 cms. long, petiole 1.0 cms. long and finely pubescent, acute, finely and sharply serrate with small dark brown glandular tipped teeth, rounded and biglandular at the base with two dark brown stalked glands; pubescent only along the midrib on the upper and on the lower surfaces, blade glabrous; stipules lobed, glandular serrate, and early deciduous. At maturity thick and firm, dark green above, paler below.
Flower	Appearing from May 1 to 15 slightly before or with the leaves, 10 to 15 mm. broad on pubescent pedicels 15 mm. in length, usually in 3 or 4 flowered umbels, sepals ovate, rounded at the apex, pinkish along the margins, pubescent; calyx tube campanulate 2 mm. deep, glandular on the inner surface, pubescent on the outer; petals white turning pinkish on fading, ovate rounded at the apex, contracted at the base into short claws; stamens 30, from 5 to 10 mm. long, glabrous, turning pinkish on fading, inserted on the calyx tube; style 10 mm. long, glabrous with truncate stigma; carpels simple, ellipsoid, glabrous.
Fruit	Ripening September 1 to October 1, or until frost overtakes it, of deep purple color, spherical or slightly oval 13 x 12 mm. and 9 x 10 mm. in the late variety, covered with a light waxen bloom, flecked with numerous light colored spots; weight .50 to 1.50 grams, flesh of a watery greenish yellow color, pulp in the late variety tending to cling to the stone and with a very bitter astringent taste, dense layer of tannin beneath the skin; stone oval, reddish in color, 8.5 mm. long and 7.8 x 5.5 mm. thick, weight .15 to .25 grams, ridged on the ventral, slightly grooved on the dorsal suture.

6. *Prunus maritima var. rubra magna*

(Large Red)

Form	A low spreading bush 3 to 6 feet high forming dense thicket like patches; branching irregular and erect, with few spinelike lateral branches. Some bushes appear quite stunted in their habit of growth.
Bark	Old stem stout, rough, cracked into thin dark gray plates which tend to persist, mostly lichen covered. Young branches smooth, dark purplish brown, numerous light

	colored lenticels, and few spine like lateral branches. Youngest twigs smooth, numerous, elongate and rather stout, puberulous, epidermis silvery gray and when shed uncovers a light reddish brown bark.
Lenticels	Numerous, small, inconspicuous, irregularly distributed over the branches, rounded and more elevated on the youngest twigs, darker and more flattened on the younger branches.
Buds	Alternate, conical shaped, sharp pointed, brown or gray, covered with numerous triangular scales, puberulous along the margins; covered with a silvery gray waxy coating which is shed in early spring. *Flower buds* lateral, more precocious in early spring, chestnut brown, rather abundantly scattered along the branches, frequently compounded with a single leaf bud near the ends of the twigs. *Leaf Buds* smaller, more pointed, dark gray and terminal, bursting after the bush is in full bloom.
Leaf Scar	Crescent shaped, with three small inconspicuous fibrovascular bundle scars.
Leaf	Ovate, alternate, 3.2 cms. wide and 6.8 cms. long, petiole 1.1 cms. long and finely pubescent; acute, finely and sharply serrate with small dark brown glandular tipped teeth; rounded and biglandular at the base with two dark brown stalked glands; pubescent along the midrib on the upper surface and along the midrib and in the axils of the primary veins on the lower surface; stipules lobed, glandular-serrate, and early deciduous. At maturity thick and firm, dark green above, paler below.
Flower	Appearing May 1 to 15, slightly before or with the leaves, 15 to 20 mm. broad on pubescent pedicles 10 mm. in length, usually in 3 or 4 flowered umbels, sepals ovate, rounded at the apex, green, pubescent; calyx tube campanulate 2 mm. deep, glandular on the inner surface, pubescent on the outer; petals white, ovate, rounded at the apex, contracted at the base into short claws; stamens 30, from 8 to 12 mm. long, glabrous inserted on the calyx tube; style 15 mm. long, glabrous with truncate stigma carpels simple, ellipsoid, glabrous.

Fruit	Ripening August 25 to September 15, of a dark red color, spherical or slightly flattened at the blossom end, 16 x 15 mm. covered with a light waxen bloom, flecked with numerous light colored spots; weight 2.40 to 3.0 grams, flesh of a watery reddish yellow color; pulp free from the stone, taste sweet, tannin less abundant than before; stone oval, compressed, reddish in color, 12.5 mm. long and 9.5 x 6.5 mm. thick, weight .40 to .50 grams, acutely ridged on the ventral, slightly grooved on the dorsal suture.

7. *Prunus maritima var. rubra parva*
(Small Red)

Form	A low spreading bush 3 to 6 feet high forming dense thicket like patches, branching rather regular and erect, with few spine-like lateral branches; bushes frequently appear gnarled and stunted in their habit of growth.
Bark	Old stem slender elongate, rough, cracked into thin dark gray plates which tend to persist, mostly lichen covered. Young branches smooth, dark purplish brown with few light colored lenticels, and few spine-like lateral branches. Youngest twigs smooth, numerous, elongate, slender, puberulous; epidermis silvery gray, persistent, when shed uncovers a light reddish brown bark.
Lenticels	Few, small, inconspicuous, irregularly distributed over the branches, rounded and more elevated on the younger twigs, darker and more flattened on the young branches.
Buds	Alternate, conical shaped, sharp pointed, brown to gray, covered with numerous triangular scales, puberulous along the margins; covered with a silvery gray epidermis which is shed in early spring. *Flower buds* more precocious in early spring, chestnut brown, rather abundantly scattered along the twigs, frequently compounded with a single leaf bud. *Leaf Buds* smaller, more pointed, dark gray and terminal, bursting after the bush is in full bloom.
Leaf Scar	Crescent shaped, with three small inconspicuous fibro-vascular bundle scars.
Leaf	Ovate, alternate, 2.0 cms. wide, 4.5 cms. long, petiole 0.9 cms. long and finely pubescent, acute, finely and sharply serrate with small dark brown glandular tipped teeth;

rounded and biglandular at the base with two dark brown stalked glands; pubescent only along the midrib on the upper and on the lower surfaces, blade glabrous; stipules lobed, glandular-serrate, and early deciduous. At maturity thick and firm, dark green above, paler below.

Flower Appearing May 1 to 15, before the leaves, 10 to 15 mm. broad on pubescent pedicels 15 mm. long, usually in 3 or 4 flowered umbels, sepals ovate, rounded at the apex, green, pubescent, calyx tube campanulate 2 mm. deep, glandular on the inner surface, pubescent on the outer; petals white, ovate, rounded at the apex, contracted at the base into short claws; stamens 30, from 5 to 10 mm. long, glabrous, inserted on the calyx tube; style 10 mm. long, glabrous, with truncate stigma; carpels simple, ellipsoid, glabrous.

Fruit Ripening August 25 to September 15, of a dark red color, spherical or slightly flattened at the blossom end, 14 x 13 mm. covered with a light waxen bloom, flecked with, numerous light colored spots; weight 1.50 to 1.90 grams, flesh of a watery reddish yellow color; pulp free from the stone, sweet, tannin layer under the skin; stone oval, compressed, reddish in color, 9.0 mm. long and 8.5 x 5.8 mm. thick, weight .25 to .30 grams, acutely ridged on the ventral, slightly grooved on the dorsal suture.

8. *Prunus maritima var. lutea magna*
(Large Yellow)

Form A low spreading bush 3 to 6 feet high forming dense thicket-like patches; branching irregular and elongate, giving the bush a rather straggling appearance as to its habit of growth.

Bark Old stem rather stout, rough, cracked into thin dark gray plates which tend to persist, mostly lichen covered. Young branches smooth, dark purplish brown, with numerous light gray lenticels. Youngest twigs smooth, numerous, elongate, slender, puberulous, epidermis very gray, persistent, when shed uncovers a light reddish brown bark.

Lenticels Numerous, small, conspicuous, irregularly distributed over the branches, rounded and more elevated on the youngest twigs, darker and more flattened on the younger branches.

Buds	Alternate, conical shaped, sharp pointed, brown to gray, covered with numerous triangular scales, puberulous along the margins; covered with a silvery gray epidermis which is shed in the early spring. *Flower buds* lateral, more precocious in early spring, chestnut brown, rather abundantly scattered along the twigs, frequently compounded with a single leaf bud. *Leaf buds* smaller, more pointed, dark gray and terminal, bursting after the bush is in full bloom.
Leaf Scar	Crescent shaped, with three small inconspicuous fibro-vascular bundle scars.
Leaf	Ovate to obovate, alternate, 3.5 cms. wide 6.8 cms. long, petiole .8 cms. long and finely pubescent; acute, finely and sharply serrate with small dark brown glandular tipped teeth, rounded and biglandular at the base with two dark brown stalked glands; pubescent along the midrib and slightly along the leading veins on the upper surface, while on the lower surface pubescent only along the midrib; stipules lobed, glandular serrate, and early deciduous. At maturity thick and firm, dark green above, paler below.
Flower	Appearing May 1 to 15 before the leaves, 15 to 20 mm. broad on pubescent pedicels 15 mm. in length, usually in 3 or 4 flowered umbels, sepals ovate, rounded at the apex, green pubescent, cayx tube campanulate, 2 mm. deep, glabrous on the inner surface, pubescent on the outer; petals white, rounded at the apex, contracted at the base into short claws; stamens 30, from 8 to 12 mm. long, glabrous, inserted on the calyx tube, style 15 mm. long, glabrous, with a truncate stigma, carpels simple, ellipsoid, glabrous.
Fruit	Ripening August 10 to 30, of a deep yellow color developing a pinkish tinge when fully mature, spherical to slightly flattened at the blossom end 17 x 16 mm. covered with a light waxen bloom, flecked with numerous light colored spots; weight 2.7 to 3.25 grams, flesh of a watery yellow color, pulp decidedly free from the stone, sweet and pleasant, tannin layer slight, much less than in the preceeding varieties, stone oval, slightly compressed, yellowish in color, 10 mm. long and 9.5 x 7.5 mm. thick, weight .35 to .45 grams, slightly ridged on the ventral, slightly grooved on the dorsal suture.

9. *Prunus maritima var. lutea parva.*

(Small Yellow)

Form	A low spreading bush 3 to 6 feet high, forming dense thicket-like patches; branching irregular and elongate, giving the bush a rather straggling appearance as to its habit of growth.
Bark	Old stem slender, elongate, rough, cracked into thin dark gray plates which tend to persist, mostly lichen covered. Young branches smooth, dark purplish brown, with numerous light colored lenticels. Youngest twigs smooth, numerous, slender, elongate, puberulous, epidermis silvery gray, persistent, when shed uncovers a light reddish brown bark.
Lenticels	Numerous, small, conspicuous, irregularly distributed over the branches, rounded and more elevated on the youngest twigs, darker and more flattened on the younger branches.
Buds	Alternate, conical shaped, sharp pointed, brown to gray covered with numerous triangular scales, puberulous along the margins; covered with a silvery gray waxy coating which is shed in early spring. *Flower buds* lateral, more precocious in early spring, chestnut brown, rather abundantly scattered along the twigs, frequently compounded with a single leaf bud near the end of the branch. *Leaf buds* smaller, more pointed, dark gray and terminal, bursting after the bush is in full bloom.
Leaf Scar	Crescent shaped, with three small inconspicuous fibrovascular bundle scars.
Leaf	Ovate, alternate, 2.5 cms. wide 5.7 cms. long, petiole 1.0 cms. long and finely pubescent; acute, finely and sharply serrate with small dark brown glandular tipped teeth; rounded and biglandular at the base with two dark brown stalked glands; very pubescent along the midrib, veins and cross veins on the upper surface, slightly less pubescent along the midrib, veins and cross veins on the lower surface; stipules lobed, glandular-serrate, and early deciduous. At maturity thick and firm, dark green above, paler below.
Flower	Appearing from May 1 to 15, slightly before, or with the leaves, 10 to 15 mm. broad on pubescent pedicels 20 mm.

long usually in 3 or 4 flowered umbels, sepals ovate, rounded at the apex, green, pubescent, calyx tube campanulate 2 mm. deep, glandular on the inner surface, pubescent on the outer; petals white, ovate, rounded at the apex, contracted at the base into short claws; stamens 30, from 5 to 10 mm. long, glabrous, inserted on the calyx tube; style 10 mm. long, glabrous, with truncate stigma; carpels simple, ellipsoid, glabrous.

Fruit Ripening August 10 to 30, of a deep yellow color, spherical to slightly flattened at the blossom end, 14 x 13 mm. covered with a light waxen bloom, flecked with numerous light colored spots; weight 1.45 to 1.85 grams, flesh of a watery yellow color, pulp decidedly free from the stone, sweet and pleasant, tannin layer slight; stone oval, slightly compressed, yellowish in color, 8.5 mm. long and 7.0 x 6.0 mm. thick, weight .25 to .35 grams, slightly ridged on the ventral, slightly grooved on the dorsal suture.

IV. Economic Value

One is immediately impressed, on seeing the vast expanse of *P. maritima* growing on the sandy soils of the Atlantic coast, and fruiting in abundance, by the thought that it should have a high economic value. The characters which would seem to warrant its horticultural possibilities are its great hardiness, late blooming, enormous productiveness, and ability to withstand adverse conditions. These were recognized by Burbank several years ago and every effort was made by him to cross it with some of the larger and finer species. This cross, however, was not effected for several years because the Beach Plum blossoms very late, long after all other plums have shed their bloom. Burbank early recognized the value of the introduction of foreign blood into the plum family, and from his hybridizing experiments—commenced in 1885 and continued up to the present time—has produced 65 varieties. Of these, 38 have been developed from Asiatic, 14 from American, and 13 from European stock.

It is interesting to note, that of the six American species of *Prunus* used in his experiments, *maritima* should have proved to be the most important, as it is the one to which the greatest interest is attached. Of the American species used all are unusually hardy. Cold does them no harm even in the most northern parts of the United States. It seems therefore that the best horticultural results can be obtained if hardy fruits are more and more developed and selected for the colder sections of our country.

The hybridizing experiments of Burbank included the crossing of the present species with hybrids of the Japenese Plum (*P. triflora*). There resulted from these crosses three interesting varieties of which the "Giant Maritima" seems to be the most marked. The fruit begins to ripen in California early in July and when ripe is of a deep crimson covered with a thin pale bloom. The flesh until fully ripe is very firm and solid, but it breaks down quickly when ripe. It is honey yellow, with a pale greenish tinge. The quality is good. The fruit is fragrant, and as large as any other plum known in 1905. It was grafted into numerous older trees and appears to be a strong grower. He states that it will never prove of much commercial value as it lacks firmness of texture. The second variety was called "East." It was not as good as he had anticipated, it was too soft for shipping, but proved to be a desirable variety for home consumption. In quality of fruit, it was probably inferior to the best Japanese hybrids. It ripens from August 1 to 15. "Pride," the third variety, is of little value as a shipping plum. It ripens too quickly so that it will not stand shipping to any great distance. It is apple shaped, dark red, a good grower, an excellent bearer and ripens about August 20th. He further states that in addition to these, nearly 2000 other promising *maritima* hybrids are now being grown from these crosses. Many of them are excellent in habit, productiveness, and hardiness and from them he hopes to introduce many new forms.

The writer has grown in the Botanic Gardens of the University of Pennsylvania *P. maritima* budded on domestic stock, which at the present time is growing very rapidly, although no blooms have appeared as yet. It is the writer's hope that interesting forms may result from proper cultivation and selection of the budded stock, and in this connection he desires to express his personal gratitude to Messrs. Hoopes Bro. and Thomas, West Chester, Pa. for their skill in effecting these graft unions for him.

This work was conducted under the direction of Professor John M. Macfarlane, of the University of Pennsylvania, to whom the writer is indebted for many helpful suggestions and criticisms, and for which he expresses his full appreciation.

V. SUMMARY

The following summarizes the body of new facts obtained by the writer during the present investigation.

1. While *P. maritima* is typically a seaside plant that grows in loose sandy soil, there are inland localized areas along the eastern sea board where it occurs. These probably represent isolated, and stranded patches of individuals, left from shore lines that once existed in previous geological periods.

2. While the average blossoming period has been determined to be the 2d of May, varieties are observed along shore lines where they are exposed to sweeping sea breezes and which do not open until May 15th.

3. The most noteworthy characteristics of *P. maritima* are shown by the writer to be its marked variation in size, in mode of branching, and in vigor of the shoots; in time of appearance, in size and in hairiness of the leaves; in size and in color of the petals, as well as in their blossoming before, during, or after the commencing expansion of the foliage leaves. The above is in line with the extreme variability of the fruits as already noted by previous observers, and would suggest that the species is undergoing marked mutational variations.

4. The flowers are shown to be andro-dioecious and this evidently affords a key to the conditions seen in autumn when some bushes are fruitless, others sparsely fruitful, and still others bear abundantly. The practical value of this observation in connection with possible future cultivation by man is evident.

5. As a rule, those bushes which bear the darker or deeper colored fruits of purple and blue tint have a general purplish color that extends even to the petals and stamens.

6. Interesting petiolar glands have been found to occur near the junction of the petiole with the lamina as well as laminar tooth-glands.

7. A concave nectary lines the interior of the receptacular cup that surrounds the pistil and this excretes a large amount of nectar.

8. The writer confirms the views previously expressed regarding the striking variability and resulting types of the fruit.

9. The extreme variation in size, shape, and weight of the stones in the different varieties has been determined and compared.

10. The variation forms in this highly variable plant recognized by the writer have been grouped under nine heads, each of which has been

given a definite varietal name, and all of these tend to appear in regions where the Beach Plum grows from Cape May Point to Cape Cod peninsula.

11. The primitive color was probably greenish yellow or greenish red. Through increasing transformation of the chloroplasts into bright chromoplasts pure yellow fruits were secured along one evolutionary line; through development of a red, purple and eventually blue color the chloroplasts became concealed, and so the climax of combined size and color evolution was reached in the large blue black.

12. Many bushes belonging to several varieties, particularly the small blue black and the small purple, are often destructively punctured by a weevil during the early stages of fruit maturation, or about two weeks after the flowering period. This produces a hardened protective secretion rich in tannin but which causes deteriotation in the quality of the fruit.

13. While the purple and the blue black fruits are often rich in tannin, the yellow fruits are comparatively poor in this.

14. The Beach Plum, like the cultivated plum, (*P. insititia*) and the Sand Cherry (*P. Besseyi*), is a mutational species, which through the agency of environmental factors, that are still hard to determine accurately, is undergoing change in individual plants, not along one but along several lines of variation.

15. The author agrees with the views put forth by Macfarlane as to the possible high economic value of the shrub for the future in its different varieties. He also records the interesting hybridizing experiments of Burbank with the present species and the Japanese Plum (*P. triflora*), as holding out hopes for origin of many new forms by hybridization.

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VII. Explanation of Plates

Plate LXVI

Fig. 1	Branching form of *P. maritima* var. *coerulea magna*, in winter condition.
Fig. 2	Branching form *P. maritima* var. *purpurea magna*, in winter condition.
Fig. 3	Branching form *P. maritima* var. *rubra parva*, in winter condition.
Fig. 4	Branching form *P. maritima* var. *lutea magna*, in winter condition.

Plate LXVII

Fig. 5	Blossoming branch *P. maritima* var. *coerulea magna*.
Fig. 6	Blossoming branch *P. maritima* var. *purpurea magna*.
Fig. 7	Blossoming branch *P. maritima* var. *rubra parva*.
Fig. 8	Blossoming branch *P. maritima* var. *lutea parva*.

Plate LXVIII

Fig. 9	Cross section of stem *P. maritima* var. *purpurea parva*, showing rings of cork and phloem. X 25.
Fig. 10	Cross section of stem *P. maritima* var. *coerulea magna*, showing elements of structure. X 25.
Fig. 11	Stipule of *P. maritima* var. *coerulea magna*, showing glandular serrate structure. X 10.
Fig. 12	Cross section of midrib *P. maritima* var. *rubra magna*. X 25.
Fig. 13	Lower epidermis of *P. maritima* var. *coerulea magna*, showing stomatic islands and conglomerate crystals of calcium oxalate. X 160.

Plate LXIX

Fig. 14	Fruits (natural size) *P. maritima* var. *coerulea magna* and var. *coerulea parva*.
Fig. 15	Fruits (natural size) *P. maritima* var. *purpurea magna* and var. *purpurea parva*.
Fig. 16	Fruits (natural size) *P. maritima* var. *praecox* showing bloom.
Fig. 17	Fruits (natural size) *P. maritima* var. *lutea magna* and var. *lutea parva*.

Plate LXX

Fig. 18 (a)	Seeds (edge and end views) *P. maritima* var. *coerulea magna*.
Fig. 18 (b)	Seeds (edge and end views) *P. maritima* var. *coerulea parva*.
Fig. 19	Seeds (edge and end views) *P. maritima* var. *praecox*.
Fig. 20 (a)	Seeds (edge and end views) *P. maritima* var. *purpurea magna*.
Fig. 20 (b)	Seeds (edge and end views) *P. maritima* var. *purpurea parva*.
Fig. 21 (a)	Seeds (edge and end views) *P. maritima* var. *rubra magna*.
Fig. 21 (b)	Seeds (edge and end views) *P. maritima* var. *rubra parva*.
Fig. 22 (a)	Seeds (edge and end views) *P. maritima* var. *lutea magna*.
Fig. 22 (b)	Seeds (edge and end views) *P. maritima* var. *lutea parva*.

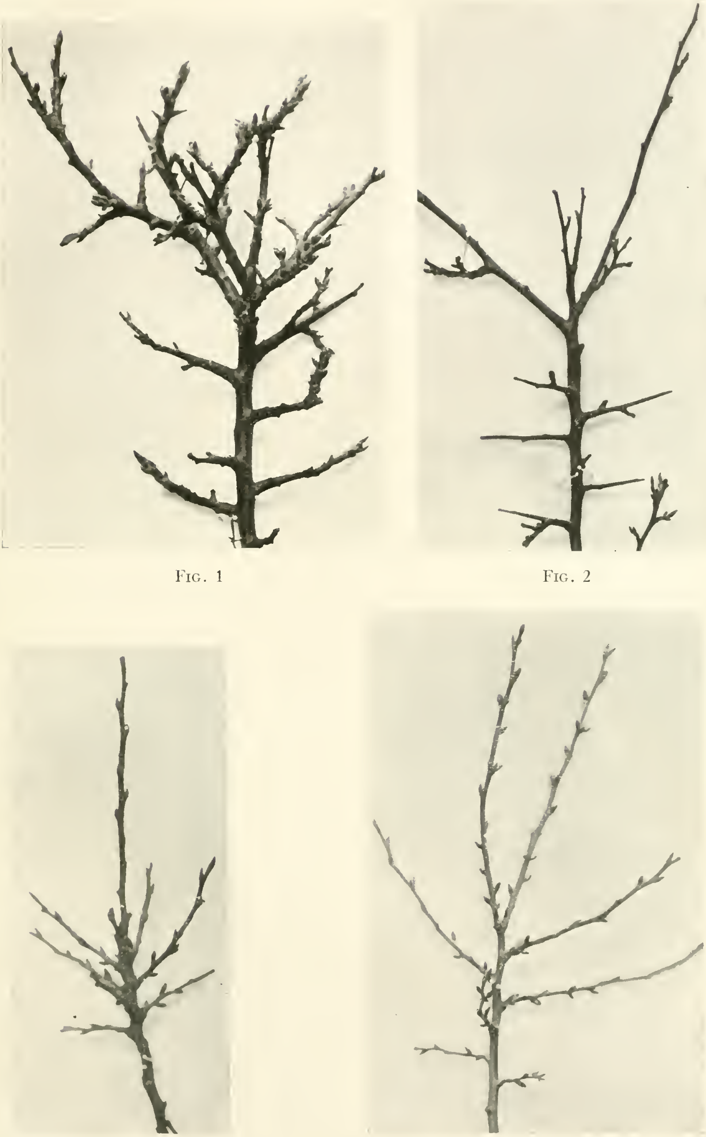


FIG. 1 FIG. 2

FIG. 3 FIG. 4

PENNYPACKER ON BEACH PLUM.

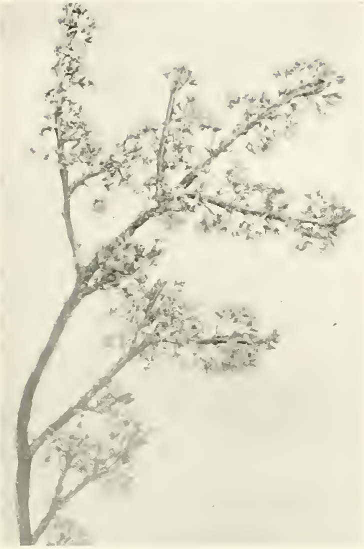
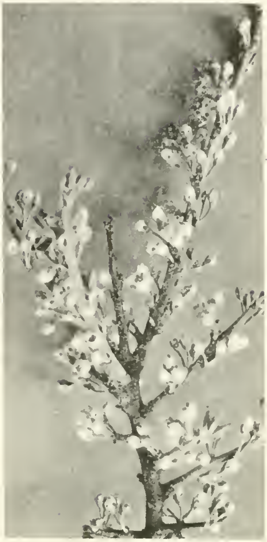


Fig. 5



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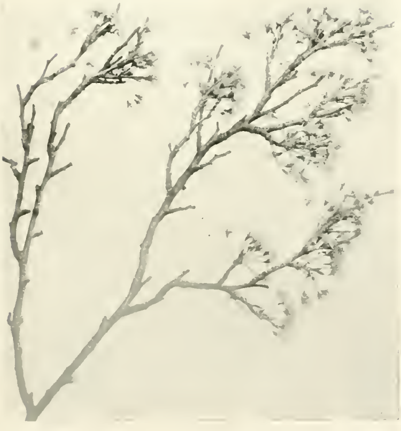

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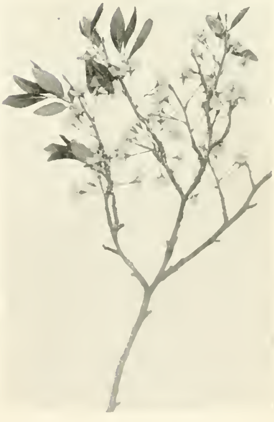

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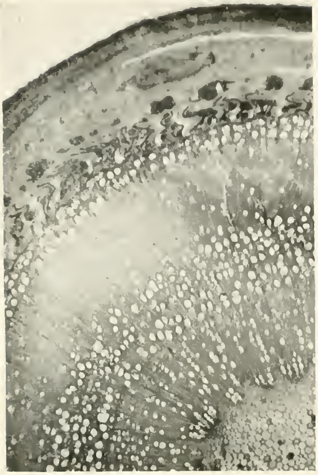
PENNYPACKER ON BEACH PLUM



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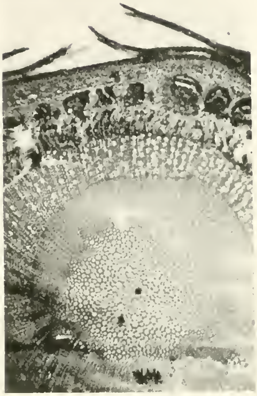

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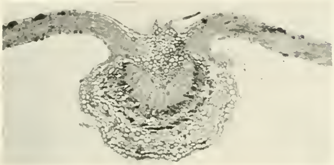

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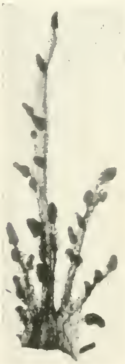


FIG. 11

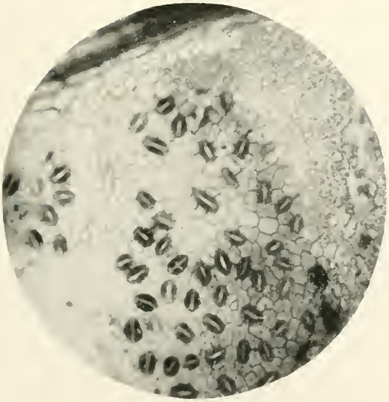


FIG. 13

PENNYPACKER ON BEACH PLUM

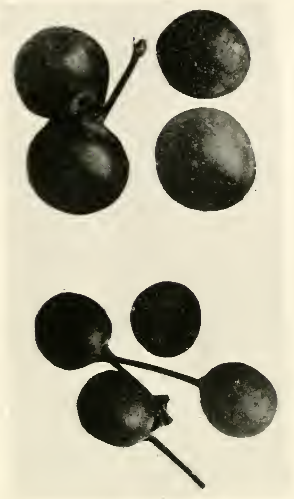


FIG. 14

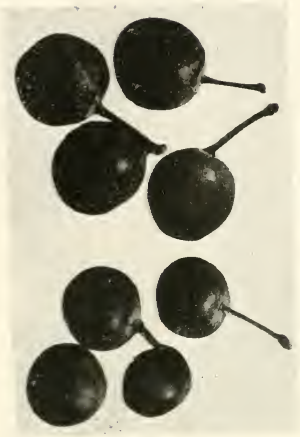


FIG. 15

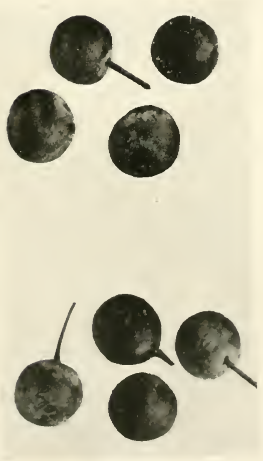


FIG. 16

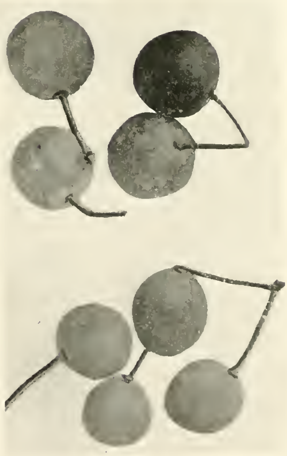


FIG. 17

PENNYPACKER ON BEACH PLUM

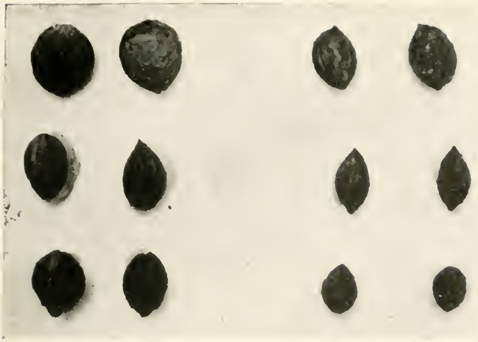


FIG. 18 A FIG. 18 B

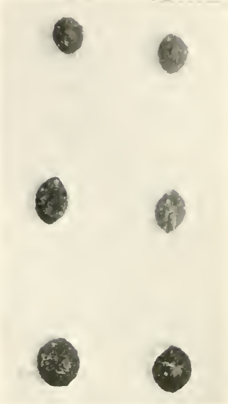
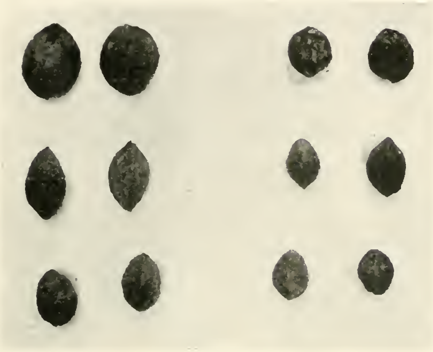


FIG. 19



A FIG. 20 B

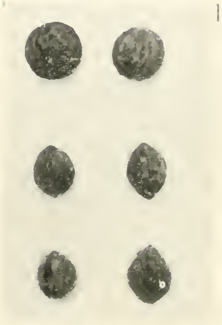
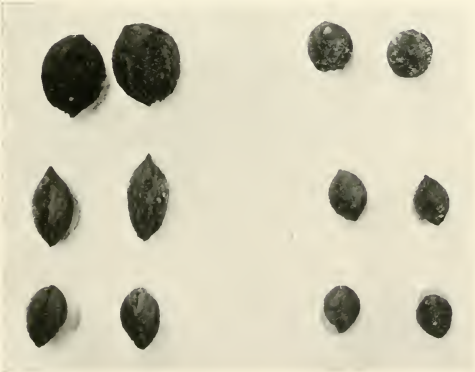


FIG. 22 A



A FIG. 21 B

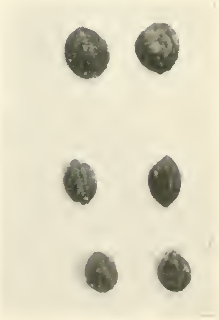


FIG. 22 B

PENNYPACKER ON BEACH PLUM

ON THE PRODUCTION OF NEW CELL FORMATIONS IN PLANTS

BY

William Randolph Taylor, B.S., M.S.

With Plates LXXI to LXXVIII

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Introduction

For five years, from 1911-1916, Dr. Caroline Rumbold studied the Chestnut Blight Disease in the Botanical Laboratory of the University of Pennsylvania, endeavoring especially to develop a method of combating the disease by the injection of chemicals into the trees. While examining microscopically the trunks of certain of these trees, she discovered that the structure of the bark in certain places differed strikingly from the normal. Further study convinced her that these abnormalities were due to the action of the injected substances and were formed in response to some unusual physiological stimulus furnished by them. At the suggestion of Dr. John M. Macfarlane, she embodied these observations in a paper (3) presented at the meeting of the American Philosophical Society held on March 3, 1916. The importance of this contribution consisted of the demonstration of unsuspected tissue-formative powers located in the soft-bast region. As Dr. Rumbold stated that she had no desire to institute further experiments to investigate the meristematic power of plant tissues, it was suggested by Dr. Macfarlane that the work be continued by the writer, to which Dr. Rumbold agreed. Work was begun about the first of April 1916, and sufficient material having accumulated to permit the drawing of just conclusions, there is here presented a summary of the experiments carried on by the writer during the past year. The writer is greatly indebted to Dr. John M. Macfarlane for suggesting this work, and for his constant supervision and encouragement while it was in progress.

Past studies of tissue abnormalities have been largely concerned with wound-healing, gall-formation and so forth. The literature on this subject has been admirably condensed by Küster (1). The major part of the work has been concerned with the general ability to produce protective wound tissue, rather than to observe the capabilities of change of each particular type of cell.

The most extensive tissue-production seems to have been obtained by Simon (5) from the ends of twigs of *Populus nigra* kept in a water-saturated atmosphere. Pustular outgrowths on leaves have been reported, and also similar intumescences on stems. The mesophyll of the leaves seems in the first case to have been the active layer. Methods of reproducing these experimentally have been found by Küster and others, and Erwin F. Smith (6) has published a paper on the production by simple chemical means of tumor-like outgrowths that extends this line of investigation. In addition to producing superficial outgrowths, he caused internal pith proliferations in the stems, these developing internal vascular structures. His experiments on this particular line he states to have been begun in June 1916. Schilberszky (4) by mechanical means produced extrafascicular bundles in *Phaseolus*. This showed that there existed in the layer of cells outside of the hard-bast tissue formative powers that under extraordinary conditions might be used by the plant to furnish itself with additional vascular tissue. These cortical cells that gave rise to the meristem were primary tissue, not secondary tissue derived from a cambium. On the contrary, the cells which developed the xylem studied by Dr. Rumbold in *Castanea* were soft-bast cells in secondary or metaphloem derived from a cambium. This is one important difference between the two conditions; another is the comparative age of the tissues involved in the two plants. It would generally be considered that the cells in the bark of a woody perennial, (in some of the cases observed several years old), would be far less responsive than those of an annual.

This notable contrast suggested that other tissues, if treated in the right way, might respond and form highly interesting structures. Something of this sort is indicated in the production of certain galls, as shown by Houard. The formation of wood from the pith has been reported by Jaccard, Prillieux, and especially Maüle. Production of bundle-cambia from non-vascular tissues is not unknown as a normal feature of certain plants.

The work of the writer on the general subject of tissue modifications may be divided into two parts. The first was an examination of a large amount of *Castanea* material injected by Dr. Rumbold, to check up her findings in that species. The second was a series of original experiments, planned to extend the observations to other plants.

In pursuance of the second part of this program, apparatus was constructed for the injection of trees, and the first experiment (on California Privet) was set up on April 18, 1916. Subsequently several species of trees were used, including *Populus carolinensis*, *Catalpa bignonioides*, *Ailanthus glandulosa*, and *Paulownia tomentosa*. These experiments did not yield very satisfactory results, due in part at least to excessive dilution of the substance injected. From the first the writer determined to conduct similar experiments on herbaceous plants, and the injection of these was begun the first week in July, 1916. It was hoped that the reactions of these would furnish the key to the very complex conditions present in *Castanea*, and the results secured from *Ricinus* cut by the end of September verified these conjectures. However, the number of plants available during the early summer was not very large, so during the winter of 1916 roots of *Polygonum Sieboldii* were started in the greenhouses and the shoots used. This plant was further experimented on outdoors the next spring (1917). Altogether, about a hundred injections of various kinds have been made, and suitable controls maintained.

Briefly expressed, the object of this study was to produce special cellular growth of all tissues capable of it, to determine what these were, and what the morphology of the products of this growth might be. In extension of the work of Dr. Rumbold, it was determined to do this as far as practicable by the injection of various chemicals in solution. As may easily be understood, it was not possible to eliminate entirely the factor of mechanical injury. The mere puncture of the needle in the work on herbaceous plants caused a slight reaction and complicated the result to some extent. Details of the behavior of the tissues are given in the descriptions of individual cases.

CASTANEA—"PARAGON"

Material and Methods

The material used in the study of tissue modifications in the Chestnut consisted of small grafted "Paragon" trees obtained from a large orchard near Martic Forge, Lancaster County, Penna. In the hope of thereby controlling the Chestnut Blight, Dr. Rumbold had injected into different trees here during 1915 and previously, a large variety of chemicals (2). On the 7th and 8th of November 1916, the writer went with Dr. Rumbold to Martic Forge and cut down a number of trees. Sections of the trunks of these were sent in to the laboratory and preserved for study.

The collection comprised trees that had been injected with various chemicals, others that had been badly infected with the blight, some in which large cankers had been carefully cleaned away a year or more previously, and others with minor injuries or normal structure for comparison. As a result of the various vicissitudes that they had undergone, many showed an extremely gnarled and twisted exterior with but little living wood, while a few were completely dead.

In all cases a hand-lens examination of the material preceded preparation for sectioning on the microtome. Each specimen was sawn thru at the point to be examined, and the surface smoothed with a sharp chisel, when examination of the surface with a good hand-lens (X 10) sufficed to show clearly the distribution and general nature of any abnormal structures in the peripheral wood or the bark. Material so prepared and coated with glycerin photographed easily (Plate LXXIV, Fig. 11). Representative specimens of the material were cut out, treated with hydrofluoric acid to remove the silica, washed, and embedded for sectioning in celloidin in the usual way.

Normal Histology of Castanea—"Paragon"

Before describing in detail the various alterations in the tissues found in the neighborhood of the wounds of *Castanea*, it is necessary that the normal structure of the stem be discussed.

The Pith in stems of the age used had long been lignified and dead. Lignification seems to occur during the first year.

The Wood consists of elongated fiber cells and of prominent pitted vessels. These latter are mostly formed in the spring, producing a distinctly ring-porous wood. The walls of these pitted vessels are not very greatly hardened, and the pits are small and transverse (Plate

LXXIII, Fig. 9). In the fibers there are pits of a special form, similar to those found in other members of this family. They are called by Solereder (7) "Bordered Pits." In form they are circular, the wall being here rather heavily thickened. The central pore is slitlike, and the direction of the slit on one side is generally the reverse of that on the other, causing under low power the appearance of a cross (Plate LXXIII, Fig. 9). This is more evident in the smaller pits, where the slits cross each other at right angles, than it is in the largest ones, the larger slits seen in these crossing more obliquely.

The Medullary Rays are very narrow, generally being but one cell wide (Plate LXXII, Fig. 5). In passing thru the bark they cross directly thru the hard-bast strands. The walls of the cells become lignified, and have many simple pits in them.

The Phloem is of two sorts. Some of the cells are soft, in cross-section somewhat rectangular, and in longitudinal section about twice as long as broad. There is a secondary differentiation of these into some that contain much tannin and into others that are comparatively free from it. The tannin may be demonstrated by treatment with ferric chloride in the usual way. In position the tannin cells may be scattered, or they may form long rows two or three cells wide and circling the stem for a considerable distance. One or two of these rows (rarely more) may lie in the ordinary soft bast between the hard-bast strands of one year and those of the year preceeding. Many of the soft-bast cells contain conglomerate crystals.

The other type of phloem or bast cell is lignified, and forms in the older regions of the bark plates or strands of hard-bast cells. These form each year a broken ring encircling the stem. The size of these hard-bast strands varies considerably, altho they are fairly uniform in thickness in any one year's ring. Around the margin of each strand is a layer of cells containing tabular crystals. Stone-cells frequently appear, and their presence cannot be attributed to any special injury to the tissues. They are not distributed in any regular manner.

The Cortex seemed to be largely gone from the specimens examined, but when it was present it showed a ring of sclerenchyma embedded in the soft parenchyma. The outer cortex in young stems consists of a collenchyma. Under the lenticels there are formed wedge-shaped areas with the cells rounded instead of rectangular as is usual elsewhere.

Cork forms early. According to Solereder the cambium originates from the outer layer of cortex cells. In the particular variety of Chestnut studied, the surface of the bark remains smooth for a number of years, but little splitting occurring. When the age of the tree or injuries necessitate it, cork-cambia form from the deeper soft-bast cells and cause the shedding of fragments of the bark.

New Tissue Formation in the Phloem of Castanea "Paragon"

In all of the specimens examined the special development of lignified tissues in the soft-bast occurred in the neighborhood of rather extensive injuries to the cambium. These injuries of course killed the bark and the cambium over a considerable area, and in addition stopped further growth from the cambium for a short distance above and below this area. This distance was never more than five centimeters, except where an injected chemical, passing up just inside of the cambium, caused a more extensive inhibition of growth. The general result did not seem to vary with the cause of the wound. The simplest conditions were those seen in trunks which were healing around a clean mechanical wound (Plate LXXI, Fig. 3). The condition after an injection was similar. Very weak solutions were ineffective. When the solutions were toxic, however, the cambium and bark adjacent to the path of the fluid were injured, became in time segregated from the healthy bark by a cork cambium, and finally dried up. Healing progressed as in the first case, the abnormalities appearing above and below the wound as before (Plate LXXI, Fig. 2). A more moderate action killed only the cambium and the innermost part of the soft-bast, in which case the abnormal tissues were formed in the bast external to the dead region.

In the region of inhibited cambial growth it was seen that the cells between the wood and the inner side of the youngest hard-bast ring eventually became lignified, largely forming wood fibers. Except in the limited regions just described, the cambium continued normal growth. Just outside the area of wound inhibition cell division was unusually active, and there was tendency to form along the border of this area a tissue growth similar to the callus that formed rapidly on the sides of the wound. The effect of this activity would be to force aside the bark over the inhibited area, unless in this area there was some means of compensating for the lack of normal cambial growth. To a great extent this is effected thru the formation of lignified patches in the soft-bast.

The simplest explanation of the origin of the complicated tissue masses produced can best be afforded thru a separate consideration of the nature of the changes in each of the tissues involved.

The Xylem or Wood was stained by the passage of the injection fluid. If the cells adjoining them are not killed by the fluid, the pitted vessels may be filled with tyloses, due in part at least, to the effect of the injection (3). Some substances caused the wood to disintegrate, killing the cambium and stimulating the formation of abnormal xylem patches external to the dead region. The medullary rays in the wood showed no particular reaction.

The Bundle-cambium as has already been mentioned, stopped growth and matured into lignified elements, mainly fibers (Plate LXXIV, Fig. 12).

The Phloem formed the greater width of the bark in trees of the age of those examined, and was the part that showed the most peculiar developments.

There resulted an active formation of lignified tissues (a) from the simple lignification of soft bast-cells, and (b) by the development from soft-bast cells of meristematic areas that subsequently formed true xylem. These meristematic areas, from their function may appropriately be termed *Xylem-cambia*. Since the formation of areas of simple lignification frequently takes place subsequent to the formation of the xylem-cambia, and in a definite relation to them, the discussion of the xylem-cambia will be given before that of the simpler case.

The formation of the xylem-cambia in the soft-bast is a condition of fundamental importance. It is interesting from the contrast it presents with *Wistaria* and other cases where a bundle-cambium originates from the pericambium, as well as with the discontinuous bundles of some of the Curvembryonae, such as the Chenopodiaceae and the Phytolaccaceae, or *Mucuna* of the Leguminosae (8). In the present case the xylem-cambium formed is not a bundle cambium, producing both phloem and xylem, but forms xylem only, and eventually is completely matured into xylem. Activity begins with the cells adjacent to the outer face of the hard-bast strands only, and proceeds progressively outward. This active zone is continually renewed on the outside by the activation of more soft-bast cells, while on the inside the cells that have been dividing mature into xylem. There was no evidence that the phloem ring of one year was more sensitive than that of any other, except that the outermost part of the phloem was never affected. This remarkable activity may continue for some time, but finally ceases. There may remain a considerable strip of unmodified soft-bast cells between the newly formed xylem patch and the hard-bast strand next outside, or this strip may become lignified as described below. In no case did the entire soft-bast between one hard-bast strand and the next outer become completely re-formed to xylem. There seemed always to be the intervening zone of lignified (or unlignified) non-xylem elements. In large and well-developed xylem-patches the distribution of elements resembles that in the year-ring of wood, the majority of the pitted vessels being inside, with the fibers on the outer face (Plate LXXIV, Fig. 12).

The elements of the xylem formed in this peculiar manner resemble those of the normal xylem very closely, differing from them in certain minor points only. They are somewhat smaller in size, and show the peculiar pit markings more prominently (Plate LXXIII, Fig. 8). Frequently they are twisted and deformed, and occasionally the pitted vessels are completely bent over at right angles to the normal. The pitted vessels also show the manner of their formation from several cells fused together end to end. These peculiarities are seen along the junction of two modified patches, where the wood forming normally around to one side of the transforming region meets and joins with that formed from the temporary xylem-cambia, and also in the first xylem formed from the new bundle cambium in the manner described below.

Before touching upon this, it is necessary that the matter of simple lignification be considered. This phase was very generally present. The cells on the inner side of the hard-bast strands were those that acted in this manner most constantly. Here the space between one hard-bast strand and the xylem area already formed on the outer face of the next inner hard-bast strand is often entirely filled by these lignified soft-bast cells. Lignification does not proceed rapidly, and may be completed in the deeper parts of the abnormal area only. Frequently there is to be seen a mixture of lignified and of unlignified elements (Plate LXXII, Fig. 6). The lignified cells are prominently pitted. Altho the tannin-filled cells do not take any initiatory part in the transformation, they as a class become more completely lignified than the ordinary soft-bast cells. The circumferential lines of these cells, lignified, may cross considerable areas of unchanged soft-bast. What has been said of these applies equally to the medullary rays of the bark, which also contain much tannin.

The soft-bast cells in the region of simple lignification show but little tendency to change their form. They appear considerably larger than the unchanged cells, but this is in part due to the shrinkage of embedding, which affects only the soft, unchanged cells.

In one place where the soft bast lignifies there does appear a change of form. The outward growth of the individual abnormal xylem areas would cause a rupture of the cells between them, unless these kept pace with this growth. This occurs in part by cell multiplication, but mainly by cell growth, and is followed by lignification.

Before passing to the closing phase of development the stone-cells may be mentioned. They were found in various locations in normal and in transforming bark, and they were seen in the patches of lignifying phloem, but not in the xylem patches forming in the phloem. They had no evident relation to these. The hard-bast strands were much dislocated, and sometimes were broken by the unequal growth of adjacent xylem-patches, but otherwise they were unmodified.

In time there arises a new bundle-cambium from soft-bast cells outside of the abnormal xylem areas. Unless there was something of this sort formed, there would be no means for growth to continue other than by the extension in number and size of the abnormal patches, and there would be no phloem formed in these regions. Instead of this happening, a meristematic zone arises on the outer face of hard-bast strands and having essentially the same origin as the xylem-cambia described above. This zone in time forms xylem and phloem (Plate LXXIII, Fig. 8) of the type ordinarily seen on the sides of a wound. Whether there originates first a xylem-cambium of the extraordinary type, which later becomes a bundle-cambium, or whether there is phloem formed from the first as well as xylem, the material at hand failed to show. Following the establishment of this new bundle-cambium, natural growth progressed as usual. There is a peculiarity of the hard-bast formed by this cambium (at least during the first year or two) that is also characteristic of that formed by the rapidly dividing cambium on the edges of a wound. The abnormal strands of hard-bast are not flat and regularly disposed, nor are they formed as in normal tissue, one ring each year, they are larger and round in transverse section, and are somewhat scattered (Plate LXXIV, Fig. 12). This, in the normal type of wound, is shown in the second figure of Dr. Rumbold's paper (3).

The abnormal development of cork-cambia that was reported by Dr. Rumbold seems, in all cases that the writer has examined, to be about what might have been expected. These cork layers cause the shelling off of bark which is seen around all wounds, and eventually over all parts of the trunk of the tree. They also occur isolated in the bark, and here are probably due, not so much to the injection, as to mechanical injuries. They may possibly be due to, and surround, little internal splits caused by strains set up by the deeper furrowing of the bark. An interesting phenomenon seen in one case was the stimulation to division of soft-bast cells and the causing of the cells so formed to line up in a radial manner around the cork-bounded cavity. When the injection caused the death of a strip of bark, this was segregated by a cork-cambium that sometimes took a peculiar step-like course, running between the hard-bast strands of the successive annual rings.

Since this work was begun as a continuation of that of Dr. Rumbold's with a view to the amplification and confirmation of her findings, it is necessary to compare the conclusions to which we have come.

The most important new point is the discovery of the same modifications in uninjected trees as she had found in injected ones. This eliminates the necessity for explaining them on any such basis as that propounded by her on page 491 of her paper (3). They are probably a normal wound-reaction of the species.

On page 486, heading 5, she states that "The wounded tissue was abnormal in that its position was reversed from the one in which it is customarily seen." So far as the writer could determine from the material at hand the direction of xylem formation from the xylem-cambia was the same as from ordinary cambia, that is, from the inner face of the cambium.

The overgrowth wound- or callus-tissue that was formed at the sides of the wounds was perfectly normal in position. In some cases the ingrowing margin was so bent over that there is produced the appearance in transverse section of a finger of wood largely surrounded by bark, and new tissue being formed on the face turned toward the trunk as well as outside. This, being merely a mechanical matter, can hardly be interpreted as a physiological reversal of orientation. As for the special xylem formed from the xylem-cambia in the soft-bast, this also the writer cannot consider to be reversed in position. A reversed relation of the xylem and phloem would imply that it was produced on the *outside* and not on the *inside* of the formative meristem, *inside* here meaning in the direction of the pith. Instead of this, it is quite evident that the meristem was outside and that the elements were produced on its inner face. A point emphasizing this is the relative position of what appears to represent spring and autumn wood in these abnormal patches, the "spring" wood being innermost.

On page 487 she states that "Quick killing was not followed by stimulation other than the formation of normal wound tissue (callus) to cover the wound." The overgrowing side callus which she used in her Figure 2 to illustrate this could hardly be expected to show the abnormal areas. The writer failed to find them here in all specimens that he examined.

In one or two cases isolated hard-bast strands were seen at the base of the callus where it joined the wood, these probably having been left behind after some little local disarrangement of the usual course of events, such as irregularities in the form of the wound. The abnormal areas might have been present close above or below the killed area and have escaped detection.

It is to be observed that the term "bark" is here used to denote all outside of the bundle-cambium. The hard-bast elements in the abnormal areas would come in time to be surrounded by wood, and, by the formation of the new bundle-cambium come to be cut off from the bark of the tree. The term "bark cambium" as used on pages 488 and 489 corresponds to the term "cork-cambium" used here. Dr. Rumbold on this latter page also states that the presence of abnormal lumps of cork visible on the surface of the trunk denoted the presence of a disturbance of the cambium and phloem tissues beneath, by which, in connection with her Figure 6 referred to, the writer understands the production of abnormal xylem patches. In the specimen indicated to the writer by her in the field as a case in point, and brought in for examination no such relationship was seen. There was merely a little superficial patch of cork.

On page 489 she says "The inhibitory effect on the cambium was transitory. In time a new cambium layer was formed, arising from phloem cells." The effect on the original cambium was as permanent as could be desired, since the cells became strongly lignified. It was only the effect on cambial growth in general that was transitory, tissue formation being taken up by the new cambium.

The last point which the writer wishes to note is Dr. Rumbold's use of the term "xylem." Thruout her paper it includes all lignified tissues except the hard-bast and the stone-cells. The writer would strongly favor limiting the meaning to those tissues that are composed of fibers, pitted vessels, etc., and which only seem to arise from a secondary meristem: that is, wood in the ordinary sense. This would exclude those areas of phloem modified by lignification without special change of cell form.

Castanea—Summary

The work on *Castanea* consisted of a microscopical examination of the tissues of trees injected with various chemicals, and of the tissues forming in the neighborhood of mechanical wounds, with a view to the determination of the exact genesis and nature of the abnormalities reported by Dr. Rumbold, their behavior during the second year of growth, and the condition of the tissues prerequisite for their formation.

As a result of an extensive wound, the growth of the normal wood cambium is stopped for a short distance above and below the wound. In the region thus affected, there may form in the soft-bast of the bark two principal types of lignified tissue not normally present there.

The simplest type consists of lignified soft-bast cells, the other is a xylem resulting from the assumption by soft-bast cells of a meristematic character.

In the latter case the first cells to become active are those on the outer faces of the hard-bast strands, and the cells external to these become involved successively.

These meristematic cells divide, and the products become matured into the elements of a xylem which closely simulates normally formed xylem.

A considerable part of the tissue between two or several rings of hard-bast strands may be re-formed in this manner over a considerable area, the patches of soft bast-cells that remain either being unaltered in structure, or the walls become lignified without marked change in the shape of the cells.

The cells between the adjacent strands of hard-bast of the same ring come to take part in the changes, and in such regions the cells become much distorted, and may appear intermediate in form between lignified bast cells and xylem elements.

The medullary rays in the region of the formation of the xylem patches grow with them and form rays similar to those in the normal xylem.

Eventually a fairly normal cambial region forming phloem as well as xylem is developed from the outermost of these meristematic areas, and, so far as the material indicated, further growth proceeded from this in much the usual fashion.

The Injection of Herbaceous Plants

Material and Methods

As has been stated, this part of the work was instituted to obtain evidence explanatory of the conditions found in *Castanea*. Naturally the first method attempted was that used by Dr. Rumbold on *Castanea* (2). The softness of the stem in the actively growing regions rendered difficult the obtaining of a water-tight connection between the stem and the tubes from the reservoir. This was partially accomplished thru the use of bolts and wing-nuts in place of the springs used by Dr. Rumbold. It only succeeded with fairly mature stems. In order to take advantage of the greater activity of young stems, another method was devised. A hypodermic syringe was used to introduce the fluid, generally into the pith cavity. After the solution was injected, the needle punctures were covered with grafting wax. As an indicator of the path taken by the injected fluid Anilin Black was used for some of the injections. For the other chemicals used, see page 285. The plants used in the experiments were *Phytolacca decandra*, *Helianthus annuus*, *Ricinus sp.*, and *Polygonum Sieboldii.* The plants were allowed varying lengths of time for such growths as might result from the injections to mature, and then were cut down. After cutting, the specimens were brought in to the laboratory and either sectioned fresh or preserved in alcohol for subsequent examination. Mounts of the Anilin Black injections were made immediately, care being taken not to extract the dye. Most of the other specimens were sectioned under alcohol, a few were embedded in Celloidin, and still fewer in Paraffin. Safranin and Methyl Green was the combination of dyes used on the unembedded material, while Safranin and Delafield's Haematoxylin served for the Celloidin and Paraffin preparations.

Summary of Experiments on Herbaceous Plants

The work on *Phytolacca* was begun early in July, 1916, and was largely in the nature of preliminary tests to determine the best methods to use on the *Helianthus* and *Ricinus* material, which was not yet ready. The gravity-method injections (2) yielded little, but the hypodermic needle gave some interesting results. The injections of Anilin Black showed that the fluid passed up thru the protoxylem region. This is what might have been expected, for the protoxylem region formed by far the largest part of the conducting tissue at the time of the injection. The only histological effect of this substance was to decrease the rapidity of xylem lignification for a few centimeters up the stem and to reduce the number of pitted vessels formed.

Picric Acid was the only other substance injected into *Phytolacca*, and as a strong solution, in small quantities. If this solution had been injected in large amounts into an internodal cavity as the weaker solutions were in later experiments, the poisonous effect would have been extreme. Internodal cavities were absent from stems of the size and age used. The shoots were not as young as those used in the other plants. The effect of the treatment was to produce a local breaking down of the tissues, pith proliferations, and a proliferation of the cortical tissues to form a wound callus. The Picric Acid was not solely responsible for the tissue growths here, since similar and nearly equal growths occurred in the control experiments, where the stems were merely punctured with a dry needle. By means of serial celloidin sections the relation of the wound to the tissues produced was followed out quite carefully in this plant.

When these experiments were planned it was arranged that the largest number should be done on *Helianthus.* In the late summer this plant became so hard in the part near the ground that it was impossible to obtain good, thin sections of it. Therefore it was possible to give much of this material only a superficial examination.

Gravity injections with Anilin Black showed the same conditions as in *Phytolacca.* In the older stems the pitted vessels as well as the protoxylem served for the conduction of the fluid.

The main injections were made with Picric Acid, and in one of the experiments this produced a very active pith-cambium (Plate LXXVI, Fig. 18 and Plate LXXVII, Fig. 23).

The work on *Ricinus* was the most successful done during the summer of 1916. This plant was found to be a favorable one by Erwin F. Smith (6). The plants used were grown from seed listed by the dealer under the names *Ricinus borboniensis arboreus, R. purpureus, and R. cambodgensis.* They were injected with Picric Acid both by the gravity and the needle methods, the latter yielding highly interesting results. The solutions in the latter case were injected into the internodal cavity and had their greatest effect on the wall of the cavity. Some of the solutions did get into the vessels, however, and caused decided changes in the tissues surrounding them.

The most notable features of the sections are, first, the well developed cambium formed in the pith, and second, the proliferations around the vascular bundles. Hardly less interesting are the proliferations of the pith cells into the internodal cavity. While preparing a set of sections of an uninjected stem, proliferations similar in character to those into the pith cavity of the injected specimens were found, altho less in extent. They were not accompanied by any evident wound to the stem, and possibly show that artificial stimulation is not requisite for their production.

A feature that was very prominent around the wound was the production of cork. This was greater in amount than that normally produced at the base of the plant. The formation of the cork occurred from a cambium that generally originated from the second layer of cortical cells inside of the epidermis. It extended for two or more centimeters on all sides of the wound.

The work on *Polygonum Sieboldii* was started at the suggestion of Dr. Macfarlane in order that advantage might be taken of its rapid growth by forcing in the greenhouses, and that injections might be made at a time of year when the plants outside were still dormant. About thirty experiments were so conducted, from January 1917 on. The first experiments outdoors were made on the 16th of April 1917, and of these fifty were eventually set up, as well as twenty controls.

Stages have been obtained showing the formation of the cambium developed from the pith cells, with the laying down of xylem by this cambium. The points shown in the other experiments:—cell-outgrowths into the pith cavity, local proliferations of cortical cells, etc., have been duplicated. A new feature appearing here for the first time in the writer's experiments is the formation of xylem from the inner cortex, which has already been reported by Schilberszky to follow his operations on *Phaseolus*. This in the present experiments only appeared when the treatment had been unusually severe.

The work on *Helianthus*, *Phytolacca* and *Ricinus* was limited to the injection of Anilin Black and Picric Acid. The choice of Picric Acid was made because it was thought to have been especially effective in Dr. Rumbold's injections. The chemicals used on *Polygonum* were:—Anilin Black, Picric Acid, Ammonium Hydrate, Lithium Carbonate, Copper Sulphate, Sodium Carbonate, Potassium Chlorate, Ammonium Carbonate, Chloroform and Urea, all in aqueous solutions and in most cases in two or more dilutions. All injections were made with the hypodermic syringe into the internodal cavity.

The action of the different chemicals differed only in rapidity and extent. The writer could trace no specificity in action, beyond the fact that some failed to produce the internal pith proliferations. There is here given a summary of the reactions in some of the more important groups of *Polygonum* injections:—

DISTILLED WATER. Both simple proliferations into the cavity and a pith cambium were present. No formation of xylem from the pith cambium was evident.

CHLOROFORM WATER. There was here an advanced formation of the pith cambium following the injection of water saturated with Chloroform.

AMMONIA. All stages in the formation of the pith cambium were found, including, in the neighborhood of the wound, the formation of xylem. Pith proliferations into the cavity also took place.

LITHIUM CARBONATE. The stages in the formation of the pith cambium, including the formation of xylem, were seen as a result of the injections made with this substance. Superficial proliferations also occurred.

COPPER SULPHATE. The pith cavity was much browned in this series and the formation of a pith cambium occurred.

PICRIC ACID. The formation of the pith cambium was active in some of this series. When the injection was so severe as to kill a part of the internode, extrafascicular cambia and bundles were produced.

The Effect of the Injection on the General Growth of the Plant

In order to fully appreciate the importance of the histological modifications produced by the injections and by the attendant wounds, it is necessary to consider the effect of these on the general health of the plant.

In general, unless the toxicity was quite high, there was no immediate effect visible on the surface of the stem. When this was the case however, the internode treated became flaccid. Later this effect extended up and down the stem and death generally followed. If a violently corrosive substance had been injected the effect would have been even more rapid.

When the substance was only strong enough to have a locally toxic effect, the first evidence of this effect was a browning around the injection holes. This gradually spread up and down the stem. This, in time, might involve three or four internodes.

The age of the shoot and its vigor at the time of the injection had considerable to do with the effect of the injection. The more vigorous and active the shoot the greater the spread of the injected fluid thru the vascular system, and also the greater the response of the pith cells. A stem that had not a vigorous habit tended to give a local reaction.

Besides these local effects, due directly to the toxicity of the injected substance, there was an effect on the growth of the injected internode that occurred even tho the solution was so weak that tissue destruction did not take place.

This was first suspected when it was found that the injected internode was shorter than those above and below it. Measurements were made to determine to what degree this took place. The degree of variation in the rapidity of elongation of the internodes depends on so many factors, such as weather, soil moisture, age and position on the shoot, that it was not possible to arrange series that did not show a great amount of variation. This indicates that great care should be exercised in the interpretation of the data. All facts considered, however, the general conclusions that have been reached are as follows:

(1) The length of the injected internode normally would be intermediate between that of the one above and the one below, but the differences between them would be slight. *This does not hold if the parts measured were too near the ground, for the internodes near the ground are much shorter than those a little higher up.*

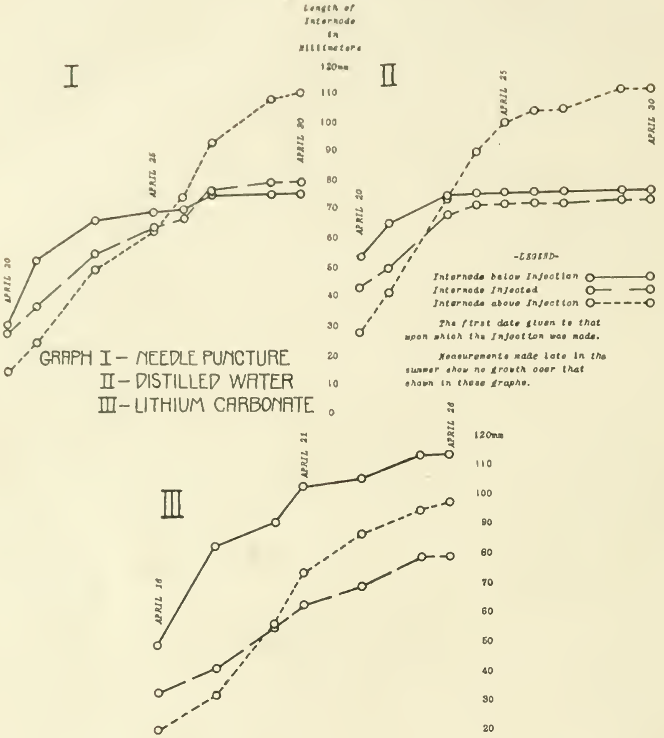
(2) After the injection there followed a short period of normal lengthening, subsequent to which the injected internode slackened growth.

(3) The internodes above and below the injected one continued to lengthen normally or nearly so. A point must here be noted that cannot be determined until the plant has been cut down and dissected. Sometimes, either thru the action of the injected substance or because of a deficiency in the internodal septum, the fluid finds its way into the internode below that injected, affecting that as well as the one originally treated.

(4) The stunting of the injected internode was permanent. No reassumption of activity tending to increase of length appeared. The diameter increase is about as normal.

On examination of the graphs one notices that the internode above the one injected lengthens rapidly, until it is of the longest of the three considered. That this is due to the effect of the injection is doubtful. The position on the stem was probably the most potent factor in causing this increase. The injected plants were not rendered sickly by the treatment; they were quite as healthy as the controls. This is only true of those where the tissue destruction was not great. One measurement was made of the plants about two weeks after the close of the regular measurements, and it showed an increase in length of the upper internode only, and that of but two to five millimeters. The point of importance in the graphs is the dwarfing of the injected internode. That there was some slight effect on the growth of the adjacent internodes was very probable, but the data are insufficent to demonstrate it.

Besides the cases used to illustrate these points, a number of additional observations were made, all on *Polygonum*.



Discussion of the Graphs—Polygonum

Needle Puncture Controls

In addition to untreated plants, a series was run on plants that had been punctured as if making an injection, and the wounds similarly protected with grafting wax. It will be seen that the punctured internode is longer than the internode below, as in the normal stem. Compare with the injected specimens. Specimens 57, 58, 59, 60, 61.

Distilled Water Injections

The injected internode is seen to be the shortest of all. The plants seem to have averaged somewhat older than the preceding. Specimens 62, 63, 64, 65, 66.

Lithium Carbonate Injections. 1:10 Saturated Aqueous Solution

This series of specimens shows a very prominent stunting of the injected internode. Specimens 33, 34, 35.

Tissue Reactions Following the Injections of Herbaceous Plants

When the injection was made, two holes were opened in the stem. The liquid, injected thru one hole, ran down the side of the cavity till this was full up to the hole left for the escape of the air. Then the excess fluid escaped thru this hole. The needle was withdrawn, the outside of the stem wiped dry, and a piece of gauze bandage smeared with melted grafting wax was wrapped around the wound. Such were the conditions when the fluid began to act on the stem. Sometimes less fluid was injected, and some of the other arrangements were different in the earlier experiments, but the general conditions were the same thruout.

It will be seen that there were two main regions upon which the substance could act:—the surface of the cavity and the tissues surrounding the vascular bundles which were cut by the introduction of the needle. That these were the only two practicable points of attack was fairly well indicated by the fact that only in these two places, the cavity of the internode and the neighborhood of the vascular bundles, was the Anilin Black seen after injection. To this there is an exception. In the neighborhood of the wound there was a considerable amount of the dye that seeped into the intercellular spaces. This seepage had no effect on the major part of the tissues. The amount of fluid that got into the vessels was much less in the hypodermic needle injections than in those where the gravity method was used, for in this latter case the number of vessels cut open was far larger.

The Epidermis. It is now necessary to turn to a detailed consideration of the tissue modifications produced by the injections. So far as the material went, there was no evidence of any tissue production from the epidermis, even tho in the young specimens injected the development of a cuticle could not have advanced very far.

The Cortex. The cortex usually only shows the effect of the injection near the wound. This may either be due to the mechanical irritation or the percolated injection fluid. The extrafascicular bundles will be considered later.

In spite of the fact that the outer cortex was a well developed collenchyma, it proliferated freely in *Phytolacca*, *Polygonum* and *Ricinus*. It proliferated in *Helianthus* also. The product was sometimes an irregular mass of cells, while on the other hand there was sometimes formed a distinct cambium. This cambium often split off cells like the most typical cork-cambium (Plate LXXVIII, Fig. 28). This is a different condition from that so often seen, where the outer layer of the cortex gives rise to the cork cambium. It was only found around wounds of injection or fissures in the epidermis resulting from mechanical injuries.

The inner cortex, which was parenchymatous, showed in all species a ready ability to proliferate. The causes which operated on the outer cortex were effective here also. The cork-like formation was present as in the outer cortex, both often taking part in the same reaction. It is improbable that much tissue could be laid down from the proliferation of these cortical cells.

The scleroid patches, when present, took no part in the reaction. No new scleroid tissue was produced.

The most prominent and interesting tissue production was from the innermost cortical layers. This formed the extra-fascicular bundles of the type mentioned by Schilberszky (4). When some great disturbance of the function of the stem occurred, such as the killing of a large part of the internode by the toxic action of the injection, the stem responded by the formation of extra-fascicular bundles. When the pith was killed, (and possibly also the xylem affected,) these bundles were often produced. In the former case as active a reaction would probably have been produced by the simple operation of removing a part of the internode, thus exposing the interior to drying. This is practically what Schilberszky did. The xylem produced was quite abnormal in appearance, the small pitted vessels being much distorted and displaced from their normal orientation. They became well lignified, however, and were quite unmistakable. These were produced in *Polygonum* alone, for only there were the solutions sufficiently strong to cause the necessary tissue destruction. The production of these bundles was limited to the neighborhood of the wounded region (Plate LXXVIII Figs. 25, 26, 27).

Schilberszky (4) describes the formation of both phloem and xylem. In the writer's preparations there was no well-defined phloem shown. Outside the xylem, and dipping into it in places, there was a soft tissue which took the Methyl Green stain readily, but it was not possible to differentiate it into a meristematic and a matured layer.

The Phloem is the next tissue to be considered. Generally it showed no response to the injections, but in at least one case of *Polygonum* where the injury had been great, there appeared a peculiar proliferation of the vascular cells. The cells that responded were apparently all from the young, unlignified cells of the phloem, cambium and probably the xylem. In this case there was a partial resumption of the formation of lignified tissue, with the production of a few pitted vessels. Unfortunately the specimen was cut too soon for much growth to have occurred, and the extent to which the cambium might have regenerated and resumed its normal function was not shown. (Plate LXXV, Fig. 16).

The Cambium was often retarded in its growth by the injection, this being especially noticeable when the fluid had passed up or down the vascular bundle. Except that they were possibly of a smaller size, the elements produced were not abnormal. See also the condition described under Phloem.

The Xylem was generally little affected by the injection, this being especially the case if the elements had become the least lignified. If the fluid passed up the protoxylem while the secondary elements were still soft, they took part in the general proliferation around the bundle. The condition is well shown in Plate LXXVIII, Fig. 29. This case was an extreme one. The cambium continued growth, forming a considerable amount of secondary wood. If secondary wood had been formed at the time of the injection, there would have been fibers and pitted vessels visible in the mass of dead xylem included in the center of the proliferated area, instead of spiral protoxylem elements only. It is to be noted that the cells which were directly around the vessels were killed by the toxic action of the fluid, and that the cells further back proliferated. This suggests strongly that the effect of the chemical was an indirect one, and that if by some mechanical means a mass of bundle and surrounding pith cells could have been killed the same result would have been produced.

The Pith was the region of the stem that gave the most interesting results in the way of tissue formation. This was in those specimens that had been injected by the needle method.

If the fluid was weak, the inner pith cells bordering on the cavity were not killed. If any reaction at all took place as the result of such an injection, it was a simple budding of the cells into the pith cavity. This condition has already been reported, and has been produced by the use of gases (6). The condition of affairs is seen in Plate LXXVII, Fig. 21.

If, now, the strength of the solution was somewhat greater, there was formed a layer of dead cells lining the cavity. From behind this, the underlying cells may bud out into the pith cavity. That the growth is not limited to the elongation of one or of a few adjacent cells is shown by Plate LXXVII, Fig. 24.

Coincident with the condition just described, there was another and more important development. This was not generally preceded by any proliferation of the pith cells into the cavity, it being strongest when the inner cells, bordering on the cavity, had been killed by the injection. It consisted of the formation of a cambioid zone from pith cells. This has been already described for *Ricinus*, but not for the other species worked upon by the writer. The procedure was as follows:—

The cells underlying the dead area first elongate radially and tend in so doing to decrease the size of the internal cavity. Then they divide, and the resulting division walls are fairly transverse to the direction of elongation (Plate LXXV, Figs. 13, 14, 15).

Next, the cells in a portion of this proliferating region divide more rapidly than the others, thus forming a meristematic zone which takes the form of a sheath often wholly surrounding the part of the cavity touched by the injected fluid. The nuclei in this meristem and in the cells of the proliferated region are often unusually prominent. The protoplasm is not dense, as in an embryonic layer in a growing point, but is scant, as in the cambia of ordinary stems.

Sometimes the cells just inside of this meristem have the appearance of having been cut off from it. As they are often yellowish, and stain readily with Safranin, it might be thought that they were cork, but repeated applications of tests for suberized membranes have failed to demonstrate its presence.

An interesting fact in this connection was found in *Polygonum*. In some of the untreated plants there had appeared deep slits or furrows in the pith. About the bases of these slits, near the protoxylem, the pith cells showed a tendency to divide, and the areas thus formed resembled the early stages in the formation of the pith-cambium.

The question now arises as to what is the true nature of the meristematic zone. Is it a true cambium, forming both phloem and xylem, or does it only form xylem? There is first formed a belt of cells that are like xylem parenchyma, there being gradually added to these in the later formed zone, pitted vessels. The formation of this zone seems to start near the insertion of the needle, and progress around the stem from there. The form of the elements is not as regular as in the normal wood, nor are they as large, but they are undoubtedly xylem elements. However, cells with thickened pitted walls were seen in some of the sections, which must be carefully distinguished from the abnormally formed xylem. They were formed from the pith cells by simple lignification, this occurrence, tho not normal to the species used, being nothing unusual. It was most apparent in a plant of *Polygonum* that had had a large part of the internode exposed to the air by the corroding action of Picric Acid introduced in the solid form.

The formation of phloem was not as clearly shown as was the formation of xylem. The cells inside of the cambium region were all so uniform in appearance (the cells formed by the original segmentation of the pith cells are to be carefully distinguished here) that there seems to be little if any true phloem present in the sections examined. It may be that a little longer time for maturation would have shown that some of the questionable regions were really phloem. The formation of phloem in this manner has already been reported for *Ricinus*, (6), by Dr. Erwin F. Smith, who also reports the formation of "concentric medullary bundles" in the pith. The writer is disinclined to assert that this pith-cambium is a true bundle cambium on the basis of his own preparations.

Herbaceous Plants—Summary

In order to obtain material that would serve to aid in the interpretation of the conditions present near the wounds in the trunks of *Castanea*, experiments were started on herbaceous plants.

These mainly consisted of the injection into the pith cavity by means of a hypodermic syringe, of various substances in aqueous solution. The injections served to stunt the growth of the injected internode.

Modifications in the tissues of the plant were produced both as a direct action of the chemical agent as a stimulating agent, and indirectly as a result of its toxic action on the cells.

The reactions that can be considered as primarily due to the first cause consisted mainly of proliferations into the pith cavity of the cells bordering on it.

Those due to the poisonous action of the injected fluid cannot always be clearly distinguished from these, nor can they always be distinguished from wound tissue reactions caused by the insertion of the injection needle.

As a result of the action of one or more of these agencies proliferations of all the tissues except the epidermis and the lignified elements were produced.

These proliferations were frequently irregular, and then from them no differentiated tissue arose.

At times under favorable conditions there was formed in these proliferated regions a cambioid zone, that laid down tissue in a manner resembling that of normal cambia.

From zones of this kind that were formed from cortical cells, cork-like protective tissues were produced.

As a result of severe injury to the stem, the innermost cortex produced xylem patches that seemed to correspond to the "extra-fascicular bundles" of Schilberszky.

The only proliferation of phloem cells that was produced was an irregular one and concerned also the cambium and probably the young xylem cells. There was a suggestion of the resumption of cambial activity in this region.

The xylem, besides being concerned in the above reaction, was able, when it had not been lignified, to proliferate.

The pith responded readily while young, and there was formed after proliferation a cambium that laid down xylem, and possibly also phloem, around the pith cavity.

CONCLUSIONS

As a result of these experiments, the writer would consider that all the elements of the normal stem are capable of extensive multiplication unless they have been modified by cuticularization, lignification, or suberization. Cells that are collenchymatous are, notwithstanding, able to proliferate freely. From these proliferated areas there may be formed cambioid zones that give rise to cork, to xylem, and possibly, to phloem. The initial multiplication may be started by mechanical or chemical means. The chemical irritation, if it suffices to cause tissue destruction, may have an ultimate effect similar to a mechanical irritation.

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EXPLANATION OF PLATES

PLATE LXXI—TYPICAL *CASTANEA* TRUNKS FROM AMONG THOSE EXAMINED

Fig. 1. The hole made for the injection may be seen at "*a*." The effect of the injection was barely visible as far up as "*b*." The saw cuts for the preliminary examination may be readily seen. Injection 32.

Fig. 2. Injected in 1914 with Lithium Hydrate 1:100 Gram-Molecular concentration. This killed the bark for a considerable distance above and below the points of injection "*c*"–"*c*." Xylem-cambial activity was visible as far as the top of the part of the specimen shown, and material for histological examination was taken from "*d*." It is shown in Plate LXXIII, Fig. 8. Injection 20.

Fig. 3. From a part of the specimen not reached by the injection. There is shown here a healing wound resulting from the removal of a blight infection. Xylem formation from xylem-cambia had occurred, showing well in material from "*e*." Injection 21.

Fig. 4. A similar case. The normal callus is shown at "*f*." The included hard-bast strands were to be seen to the top of the part of the specimen figured. Injection 31.

PLATE LXXII—THE PHLOEM, NORMAL AND MODIFIED—*CASTANEA*

Fig. 5. Normal Phloem—Transverse Section. The hard-bast of two rings is shown at "*a*." The dark row of cells that surrounds each of the hard-bast strands is the layer of crystal cells. The soft-bast, "*b*," fills all of the space between the hard-bast strands. The medullary rays are marked "*c*," the tannin-containing soft-bast cells "*d*." Three of the rows that these form are shown, the lowest only being marked. X 50.

Fig. 6. Injected in 1916 with Sodium Hydroxide, 1:1000 G.M. This shows, in transverse section, phloem in the process of lignification. The growth in this specimen was not sufficiently active to produce pitted vessels. In this figure "*i*" indicates a medullary ray. A narrow hard-bast strand is marked "*j*"–"*j*"; the cells did not stain heavily and therefore do not show clearly. They may be distinguished by the dark central spot caused by the refractivity of the wall. The lignified phloem cells, "*l*," may be distinguished by their thick walls, the unmodified cell walls appearing dark, but much thinner. These are shown at "*m*." Three of the rows of tannin cells may be seen, the uppermost passing thru the uppermost "*i*." Injection 28. X 180.

Fig. 7. Lignified Phloem, Longitudinal Section. This is a tangential section, showing at "*o*" a medullary ray in end view. Injection 30. X 130.

PLATE LXXIII—XYLEM FORMED FROM THE BUNDLE-CAMBIA—*CASTANEA*

Fig. 8. Longitudinal Section of Xylem from the New or Re-formed Bundle Cambium. The hard-bast strand, outside which the xylem was formed is seen at "*h*." The wood fibers appear at "*d*." A medullary ray has been marked "*e*." At "*f*" is shown one of the pitted vessels, the wall of another shows at "*g*." Injection 20. X 50.

Fig. 9. The Bordered Pits of the Normal Wood Fibers. Longitudinal Section. The field shown here was an unusually favorable one for the demonstration of these pits. The round area about the pore is shown. X 610.

Fig. 10. The Structure of an Individual Xylem Patch. At "*a*" is located a hard-bast strand. The direction of the pith is toward the bottom of the sheet. It will be easily seen that the xylem is continuous outward from the hard-bast, and that the pitted vessels are mostly in the earlier formed wood, as in the normal stem. The darker area "*b*" outside of the xylem patch is the region of lignified phloem cells. The lignification here is completed, no cambial zone remaining between the xylem and the lignified phloem. Injection 28. X 75.

PLATE LXXIV—XYLEM PRODUCTION ORIGINATING IN PHLOEM TISSUE—*CASTANEA*

Fig. 11. Appearance of the Modified Regions when viewed as Opaque Objects. When dealing with a wood as hard as that of *Castanea*, it is advantageous to be able to recognize the important features without resorting to the preparation of microtome sections. This photograph shows part of a large area as it would be seen if prepared as described on page 274 and viewed with a high-power hand lens. It is difficult to show the hard-bast strands

in such a photograph as this, they are too similar in color to the surrounding soft-bast. In the photograph "*c*" is just outside the region of the reformed cambium. A little below and to the right of "*c*" there is a lighter patch separated from the xylem by a dark line, and other smaller patches are to be seen in similar positions. These are hard-bast patches corresponding to "*g*" of Plate LXXIV, Fig. 12. The included hard-bast strands are marked "*d*" and "*e*." The group of hard-bast strands of which "*e*" is a part is not of the same appearance as the more normal shaped ones ("*d*") farther out. This irregularity was caused by an injury to the cambium preceeding the removal of the blight infection, which subsequent operation in turn caused the formation of the xylem cambia. From "*f*" downward is wood formed from the old cambium before its growth was stopped. X 11.

Fig. 12. General View of a Transformed Area. It is very difficult to show the complex details of the transformed areas in a low-power photograph. In the illustration all above "*h*" is normal phloem. Just below "*h*" there is a little local disturbance of the growth. The reformed bundle-cambium stretches across the picture between the phloem and the somewhat darker xylem. Just outside of it are located ("*g*") the hard-bast patches formed from this cambium. At "*j*" is the row of hard-bast strands outside of which the new bundle-cambium started activity. At "*i*" is the only hard-bast ring which had formed outside of it the temporary xylem-cambia. X 14.

KEY TO THE LETTERING OF THE FIGURES OF THE HERBACEOUS PLANTS

(Other signs than these are explained in the individual descriptions.)

"*cp*"	Parenchyma of Cortex	"*mx*"	Metaxylem of Wood
"*hb*"	Hard-bast of Phloem	"*p*"	Pith

PLATE LXXV—THE PITH CAMBIUM IN *POLYGONUM*

The first two of these figures hardly need any explanation: they show the pith region just bordering on the cavity, and the increase in the number of the cell walls laid down in the formation of the pith-cambium. The description of this process is given on page 292.

Fig. 13. First Stages in the Formation of the Pith Cambium. From Injection No. 3, where Picric Acid was introduced in solid form into the treated internode. X 55.

Fig. 14. Well Formed Pith Cambium. From injection No. 1, Distilled Water. X 55.

Fig. 15. This is a general view of one of the pith regions and part of the bundle ring in Injection No. 1, and shows the point of entrance of the injection needle. The letters "*f*"–"*f*" mark the position of the pith cambium. X 9.

Fig. 16. Proliferation in the Phloem-Cambium Region, *Polygonum*. From Injection No. 6, where Calcium Nitrate in solid form was introduced into the treated internode. This figure and the next show two phases of the same action. In this one there are two lines of activity, one along "*b*"–"*b*," the other more external at "*d*"–"*d*." The cells in between were not in an active condition at the time the plant was cut down. At "*f*" there is a group of wood elements, probably pitted vessels, that have most likely been formed from the innermost cambial zone. X 55.

Fig. 17. Xylem Formation from the Pith Cambium. Because of excessively deep staining the details of this preparation were somewhat obscured. At "*a*" is seen a large pitted vessel. The axis of the vessel is at right angles to the axis of the stem. This is often the case near the wound. The active zone was located at about "*b*"–"*b*." The pith cavity is above "*d*," where the innermost cells are dead and stained dark. The more normal pith is seen to the left. From Injection No. 12, where 1% NH_4OH was used. X 55.

PLATE LXXVI—THE MAXIMUM DEVELOPMENT OF THE PITH CAMBIUM

Fig. 18. *Helianthus*. This specimen was taken from Injection No. 7, treated with 1:1000 G.M. Picric Acid. It shows the point of entrance of the needle. There was no pith cavity formed. There was a considerable reaction here, and the pitted vessels formed from the pith-cambium generally had their axes parallel with that of the stem. The large cell shown at "*a*" is not a pitted vessel with a horizontal axis, but one of the pith cells that, though it elongated, did not divide. See also Plate LXXVII, Fig. 23. X 11.

Fig. 19. *Ricinus*. From Injection No. 1, injected with Picric Acid, 1:100 G.M. Here, as in *Helianthus*, the internode was nearly solid at the time of the injection, and the little cavity that originally existed was filled by the multiplication of the pith cells. The xylem shows as a dark band, and is marked "*b*." The split caused by the needle is marked "*d*." X 11.

PLATE LXXVII—PROLIFERATIONS INTO THE PITH CAVITY AND PHLOEM-CAMBIUM PROLIFERATION

Fig. 20. *Ricinus* Pith Proliferations into the Cavity. From Injection No. 1a, treated with 1:100 G.M. Picric Acid. Note the tuberculate appearance of the proliferated cells. The dark masses ("*a*") at the bases of some of the enlarged cells are dead cells. X 55.

Fig. 21. *Polygonum* Pith Proliferations into the Pith Cavity. From Injection No. 9, treated with 1:50 G.M. Picric Acid. This specimen shows a moderate development of the pith cells. The ends of the cells do not show the tuberculate appearance that was developed in *Ricinus*. Below the enlarged cells there may be seen the beginning of a pith cambium. X 55.

Fig. 22. This shows a type of proliferation in *Ricinus* that involves young metaxylem as well as pith. Below "*mx*" is shown metaxylem that has not been affected. Compare with Plate LXXVIII Fig. 29. X 45.

Fig. 23. The Xylem Formed from the Pith Cambium in *Helianthus*. From Injection No. 7, treated with 1:100 Picric Acid. The character of the pitted vessels formed from the pith-cambium is hard to show. The wall of a pitted vessel is shown at "*g*." X 85.

Fig. 24. Proliferation of a Large Mass of Cells in *Polygonum*. From Injection No. 19, treated with NH_4OH 10% Solution. This shows that not only can the superficial cells proliferate singly or in small groups, but large masses of deep-lying cells can act together and form nodules on the sides of the cavity. X 17.

PLATE LXXVIII—THE FORMATION OF THE EXTRAFASCICULAR BUNDLES IN *POLYGONUM*—ALSO PROLIFERATIONS AROUND THE PROTOXYLEM AND CORK FORMATION IN *RICINUS*

Fig. 25. The First Stage in the Formation of the Extrafascicular Bundle. From Injection No. 23, see above. There are seen here the first cell walls laid down in the formation of an extra-fascicular bundle. Note especially "*a*." X 55.

Fig. 26. A Well Developed Extrafascicular Cambium, *Polygonum*. From Injection No. 23, see above. The cambium shown here had just begun to lay down wood when the specimen was cut. There is seen a pitted vessel at "*b*." X 55.

Fig. 27. Extra-fascicular Bundle in *Polygonum*. This shows one of the most advanced cases that these experiments have produced. A large group of pitted vessels is shown at "*d*." X 70.

Fig. 28. Cork Formation in *Ricinus*. From Injection 1b, for data see Injection 1a, above. This photograph shows the manner of cork formation in *Ricinus*. The interest attaches to the manner in which the different layers of the collenchymatous outer cortex take part in the process. The needle hole was above the top of the picture. It is easy to see the individual layers of cells, as "*a*," "*b*," "*d*," along the lower margin of the field, and to trace each row upward and determine what part it had in the formation of the cork-cambium. The position of this cambium is along "*f*"–"*f*." It will be noted that the nearer the region under observation is to the puncture, the deeper are the layers that take part in the cork formation. X 45.

Fig. 29. An Advanced Case of Proliferation in *Ricinus*. From Injection 2c, treated with Picric Acid 1:100 G.M. The tissue destruction here was much more considerable than in the preceeding case. Considerable numbers of dark conglomerate crystals are seen that are located in a tissue that must have come from the cambium, either directly or by proliferation of cells previously formed from it. The cambium had resumed normal growth, and the normal metaxylem is seen near the lower part of the picture. X 20.

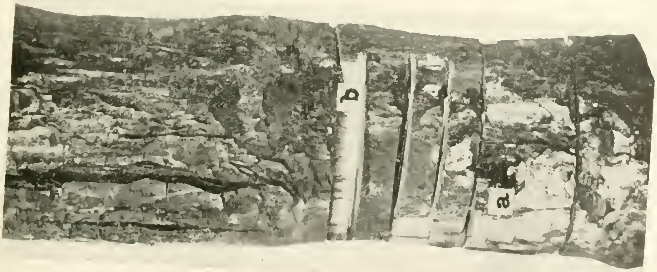

Fig. 1

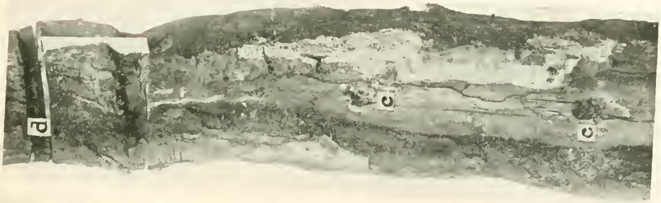

Fig. 2

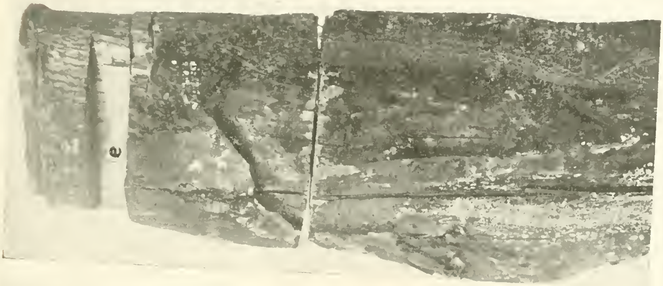

Fig. 3

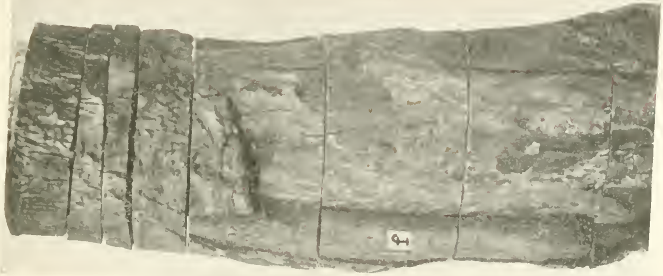

Fig. 4

TAYLOR ON CELL FORMATIONS

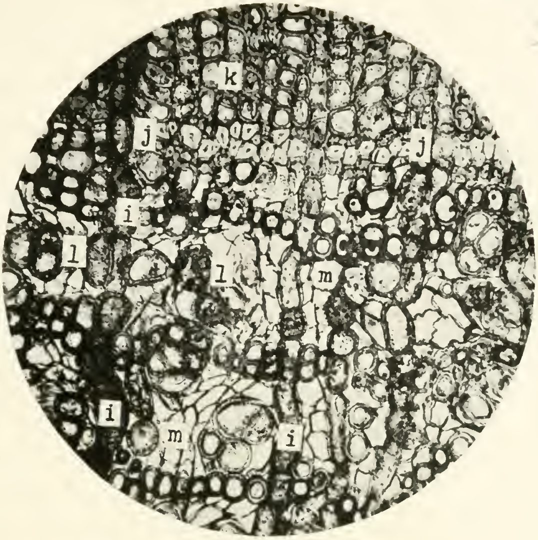

Fig. 6

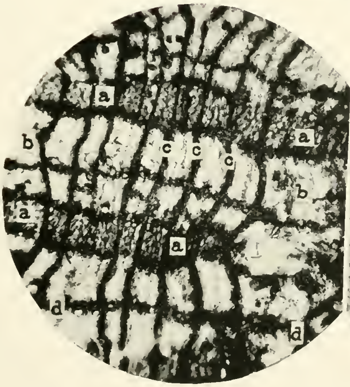

Fig. 5

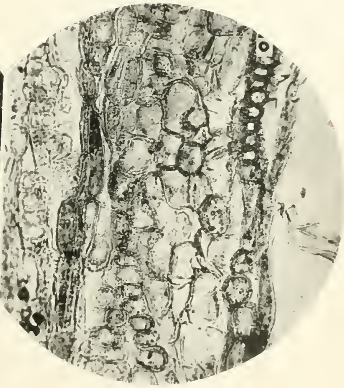

Fig. 7

TAYLOR ON CELL FORMATIONS

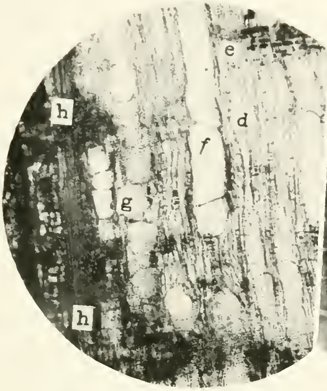

Fig. 8

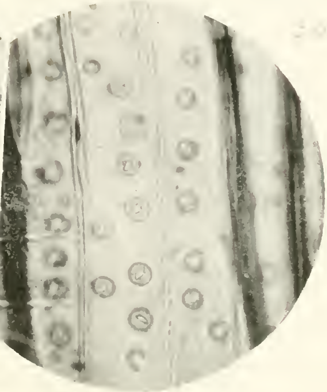

Fig. 9

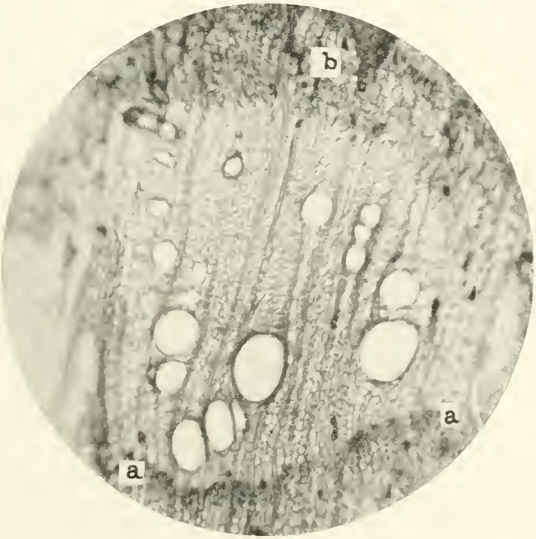

Fig. 10

TAYLOR ON CELL FORMATIONS

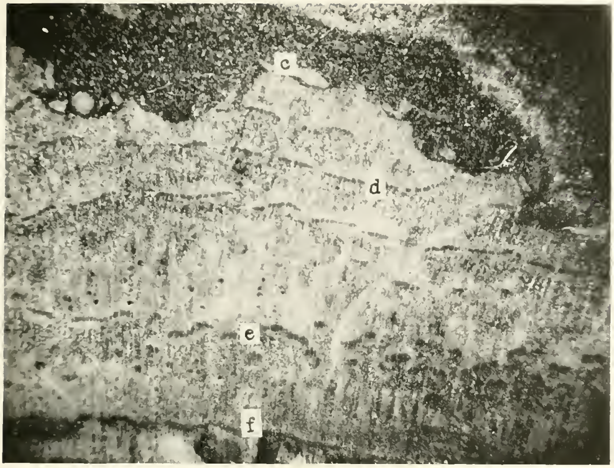

FIG. 11

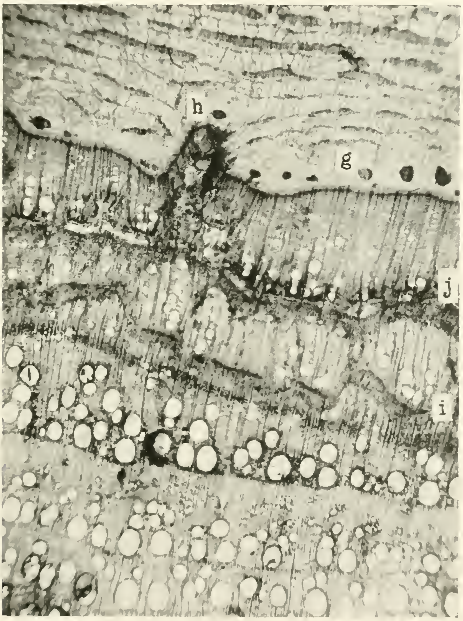

FIG. 12

TAYLOR ON CELL FORMATIONS

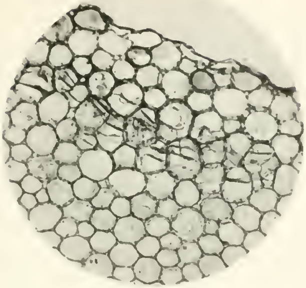

Fig. 13

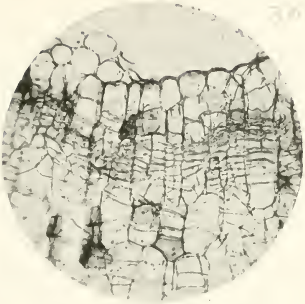

Fig. 14

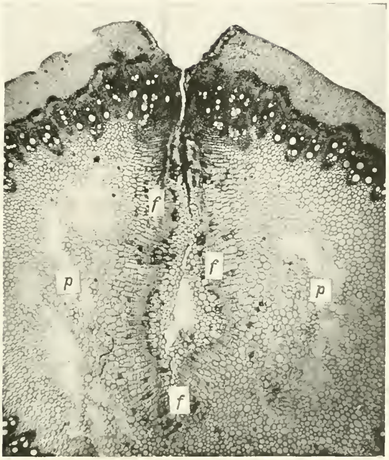

Fig. 15

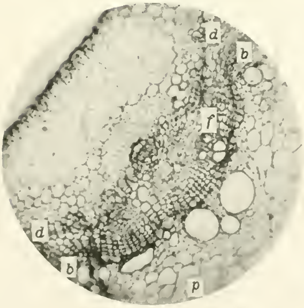

Fig. 16

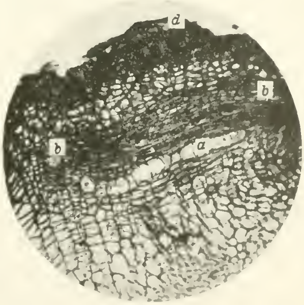

Fig. 17

TAYLOR ON CELL FORMATIONS

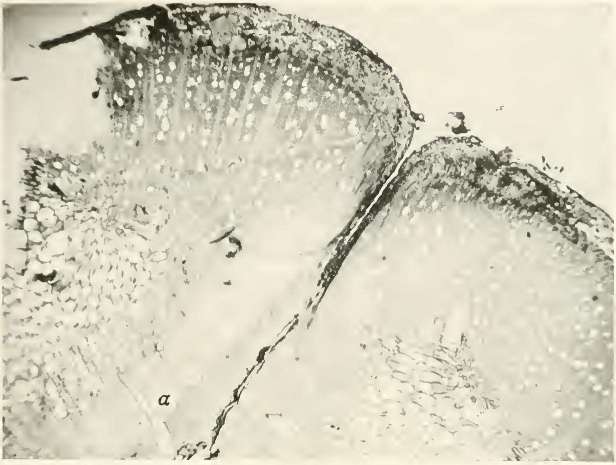

Fig. 18

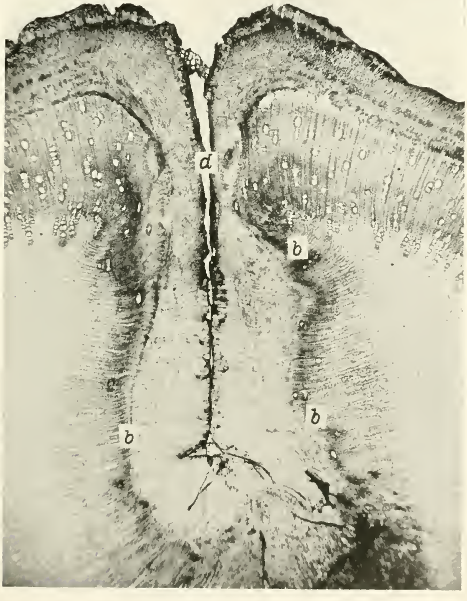

Fig. 19

TAYLOR ON CELL FORMATIONS

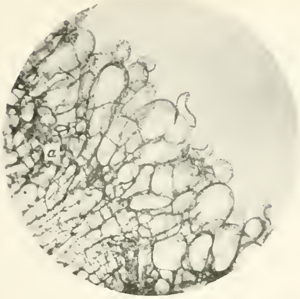

FIG. 20

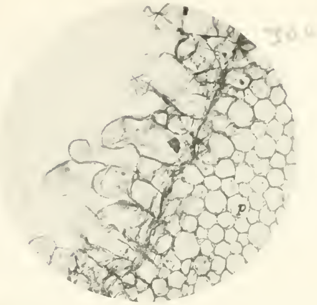

FIG. 21

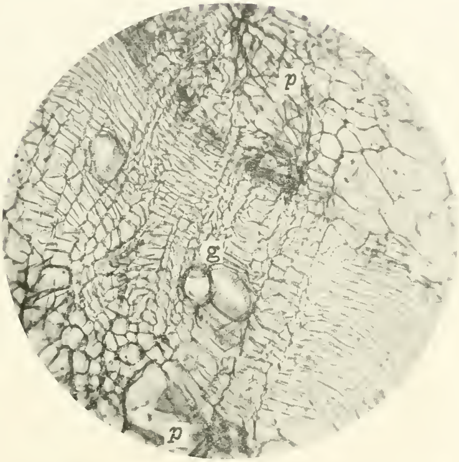

FIG. 23

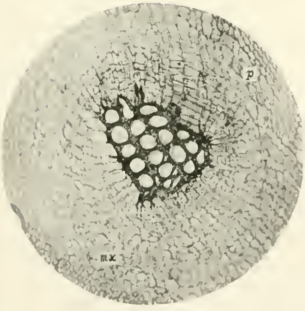

FIG. 22

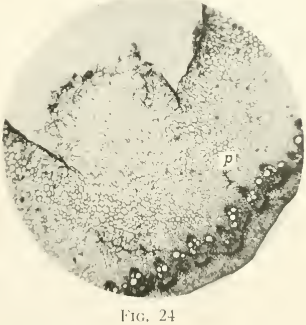

FIG. 24

TAYLOR ON CELL FORMATIONS

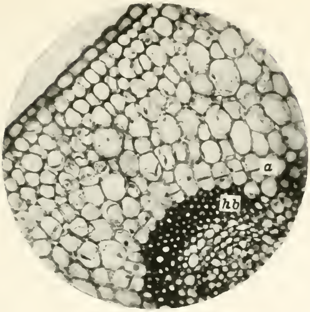

Fig. 25

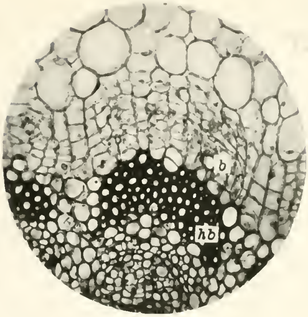

Fig. 26

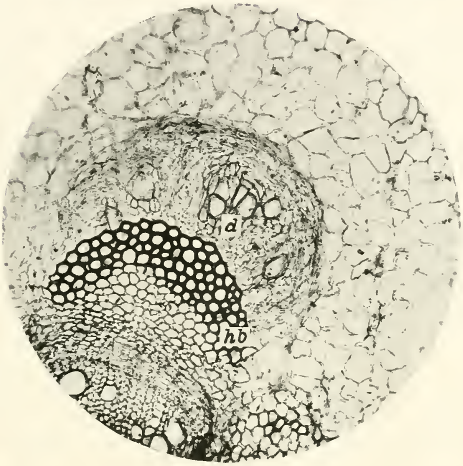

Fig. 27

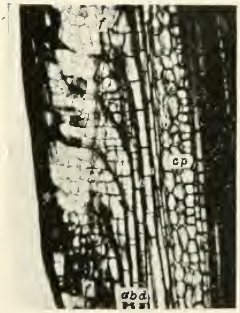

Fig. 28

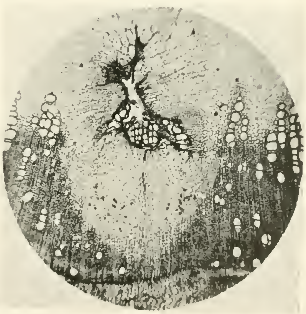

Fig. 29

TAYLOR ON CELL FORMATIONS

THE PEANUT (*Arachis hypogaea*)—ITS HISTORY, HISTOLOGY, PHYSIOLOGY, AND UTILITY

BY

Ralph Augustus Waldron, B.S., M.S.

With Plates LXXIX and LXXX

Thesis Presented to the Faculty of the Graduate School of the University of Pennsylvania, May 1918, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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INTRODUCTION

Few plants present as great interest or diversity of problems for botanical study as the peanut. Agriculturally it is of great importance as a soil renovator and forage plant. From an economic standpoint, its products are of great value, every part of the plant being of some direct or indirect use. The peanut, potato, cotton, tobacco and Indian corn,—five plants which are exerting great influence in the world's commerce and industries—were contributed by the new world. The peanut, although still in the background of some of these, promises to rival, if not surpass, them in importance. It is often grown in the economic house of botanical gardens and as a novelty out of doors. After a brief botanical study of the plant from the morphological standpoint, certain problems were presented which bear an important relation to its physiology, and are rather striking in their bearing on some well known ecological problems. A study of the history of the plant, and brief discussions concerning its economy in nature and its utilization by man, have now been taken up in succeeding pages. The writer, in here recording these observations, feels that he has made but a beginning and hopes to continue investigations in the future.

The writer wishes to express his grateful appreciation to Professor John M. Macfarlane, of the University of Pennsylvania, for his many suggestions and kind guidance in working up this treatise.

HISTORY

Introduction. With few exceptions, authors agree that the original home of the peanut (*Arachis hypogaea*) is uncertain. In the mind of the writer, there are sufficient facts at hand to state definitely, as have one or two already, that it is a native of Brazil, although, as with many other extensively cultivated plants, it has never been recognized in the truly wild state. There is no evidence to contradict the view that it is a native of this part of South America. The writer gives below a synopsis of his studies regarding the native home of the plant and its history so far as known in relation to man.

Early American Records. The earliest mentions in any existing literature are those pertaining to Brazil and Peru, and these antedate any found in European works. Acosta[1], in his work published in 1598, refers to it along with other plants which are native to Brazil, and calls it "mani," a name still applied to it among Spanish speaking people of South America. Monardes[2], according to Marcgraf and Piso[3], indicated its presence in Peru about this time, giving it the name of "anchic." Aside from these and other early mentions in literature, fruits of the plant were found in tombs at Ancon, Peru. Their presence there undoubtedly antedates the Spanish conquest, and so, also, any written record. According to Dubard[4], it was taken from Brazil to Peru sometime before the sixteenth century and there "was cultivated from an early unknown date." Among European works, Parkinson[5] in his celebrated "Theatrum Botanicum" published in 1640, gives an illustration of the fruit, which is very likely the first. A few years later, (1648) Marcgraf and Piso were the first to figure the whole plant (Fig. 1). It seems worth while to quote parts of Parkinson's quaint description as follows:

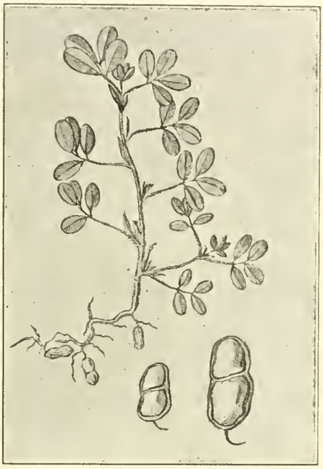

Figure 1 (after Marcgraf & Piso). Mundubi Braziliensis.

"ARACHUS *υπογειισ* AMERICANUS. UNDERGROUND CICHELING OF AMERICA OR INDIAN EARTHNUTS."

"The Indian Earth-nuts (the figure whereof, I give you together as they are termed to us by them that have brought them us) are very likely to grow from such like plants as are formerly described, (Species of *Vicia*) not onely by the name but by the sight and taste of the thing it selfe, for wee have not yet seene the face thereof above ground, yet the fruit, or Pease-cods (as I may so call it) is farre larger, whose outer huske is thicke and somewhat long, round at both ends, or a little hooked at the lower end, of a sullen whitish color on the outside, striped, and as it were wrinkled, bunching out into two parts, where the two nuts (for they are bigger than any Filberd kernell) or Pease doe lie joyning close one unto another, being somewhat long, with the roundnesse firme and solide, and of a darke reddish colour on the out side, and white within tasting sweet like a Nut, but more oily."

Concerning the introduction into Europe Parkinson's discussion indicates that they were introduced into Portugal. He received specimens sent from Candy and Lisbon. To quote him further he states that the Indian earthnuts are found in "most places of America, as well as to the South, as West parts thereof, both on the Maine and Islands; and generally called by our English Sea-men that goe into those parts Earth-nuts, erroneously enough, as they do most other things that they there meete with."

Eight years after Parkinson's reference the plant was described as follows by Marcgraf and Piso—"Mundubi Brasiliensis Herba, in pedalem aut bipedalem altitudinem adsurgit, caule quadrato aut striato, ex viridi ruffescente & piloso. Hinc inde enascuntur ramuli primo quasi caulem amplectentes & foliolis angustis, acuminatis stipati; mox habent nodum ac trium vel quatuor digitorum longitudine extenduntur; continetq; quilibet ramulus quatuor folia, duo semper sibi opposita paulo plus quam duos digitos longa sesquidigitum lata superne, laete viridia, instar trifolii, inferne paulum canescentia, nervo conspicuo & subtilibus venulis quasi parallelis dotata, raris quoque pilis vestita. Ad exortum ramulorum qui folia gerunt prodit pediculus sesquidigitum circiter longus, tenuis, flosculum gerens flavum & per oras rubentem duobus foliolis constantem, more viciorum aut trifolii. Radix illius haud longa, tenuis, contorta, filamentosa, cui adnascuntur folliculi ex albicante grysei, figura minimae cucurbitae, oblongae, magnitudinae Myrobalani fragiles: quilibet autem continet in se duos nucleos, pellicula saturate purpurea vestitos, carne intus alba, oleaginosa, sapore pistaceorum, qui comeduntur cocti & inter bellaria aponuntur. Multum tamen comesti capitis dolores causare ajunt. Fructu integro quassato nucle intus strepunt"

Linnaeus[6] says it inhabits Surinam, Brazil and Peru, but does not state whether it is wild or cultivated.

Lack of Evidence as to its extra-American Origin. Among old world literature antedating the 16th century, no mention is made. It was thus unknown there before the discovery of the new world. According to Watt[7], all Greek, Latin, Bengalese and Arabian writers are silent concerning the plant. This is very significant since the peanut is too valuable a plant to have been known to Sanskrit speaking peoples and not be used by them. Such a plant could hardly have an antiquity among them without some record being kept. Until quite recently a mention by Theophrastus of an Egyptian grown plant was thought by some, to be a reference to *Arachis*, but this has since been disproved. If it had ever existed in Egypt it could still be found there. Furthermore no mention is made of it in the works of Forskal[8] or Delile[9]. It is not recorded by any early writer on the flora of India. According to De Candolle[10] in Dr. Bretschneider's study of Chinese works[11], the statement is made that its introduction into that country was in the *sixteenth* century. It is not mentioned in ancient Chinese literature. This suggests the possibility of its introduction there from Peru by such expeditions as Magellan's.

De Candolle states: "The antiquity of its cultivation in Africa is an argument of some force which compensates to a certain degree its antiquity in Brazil." The only points offered to indicate an African antiquity are (1) the statement by Sloane[12] that it was used as food on the early slave ships sailing between Africa and America, and (2) that it now has a wide area of cultivation there, both of which could very readily have occurred after its introduction from Brazil. The writer would suggest that the very earliest ships to sail from Brazil to Africa took seeds of *Arachis* to that place, and the environment of the west coast being ideal for its growth, its cultivation very early became widespread; and this has, during the last century, developed into a great industry in the French colonies.

Pison, in his early Brazilian work figures a somewhat similar plant, in its habit of fruit production, to *Arachis*, but states it to be African, while he says *Arachis* is Brazilian. This was a species of *Voandzeia*. To quote De Candolle again, he states, "the silence of Greek, Latin and Arab authors, and the absence of the species in Egypt at Forskal's time lead me to think that its cultivation in Guinea, Senegal, and the east coast of Africa is not of very ancient date; neither has it the marks of a great antiquity in Asia. No Sanskrit name for it is known, but only a Hindustani one. Rumphius[13] says that it was imported from Japan to several islands of the Indian Archipelago. It would in that case have borne only foreign names, like the Chinese name, for instance, which signified "earth bean." At the end of the last century (19th) it was generally cultivated in China and Cochin-China. Yet, in spite of Rumphius's theory of an introduction into the islands from China or Japan, I see that Thunberg does not speak of it in his Japanese Flora. Now, Japan has had dealings with China for sixteen centuries, and cultivated plants, natives of one of the two countries, were commonly early introduced into the other. It is not mentioned by Forster among the plants employed in the small islands of the Pacific. All these facts point to an American origin." No authors speak of it wild or uncultivated in either hemisphere. Those who speak of it in Asia or Africa carefully say it is cultivated. Piso, in writing of Brazil, says the species is planted. Marcgraf does not mention it as cultivated, indicating however that it may have been. As to foreign names such as the Chinese, meaning "earth-bean," they are all such as would occur to any one upon seeing the plant. Contrary, therefore, to the suggestion of some authors, little significance need be attached to the American names not having accompanied it in its travels to Japan and the Orient.

According to Watt, Sir George Birdwood in his Bombay products gave it a Sanskrit name meaning earth-gram. This name has been repeated by some subsequent writers without the authenticity of it being inquired into.

Distribution. According to Bentham[14] in "Flora Brasiliensis," there are seven species of *Arachis* found in Brazil, six of which are found in the wild state. The other (*A. hypogaea*) he states is generally cultivated in all warm parts of the world. Now, De Candolle well remarks in this relation that: "A genus of which all the well known species are thus placed in a single region of America can scarcely have a species common to both hemispheres; it would be too great an exception to the law of geographical botany." By referring to the accompanying outline map (Figure 2) which shows the reported distribution of these species it will be noted that *A. prostrata* is the most widespread, and *A. pusilla* the most restricted of the group.

The writer asks if varieties of this plant, so generally grown in such environment as exists in Brazil, may not be cultivated forms of one or more Brazilian species?

Of the several cultivated varieties grown today there are recognized two general types of plants as follows: (1) The bunch type, growing erect and bearing its fruit around the base of a single stem. The Spanish variety is an example. It can withstand considerable moisture conditions and its erectness suggests a shade loving tendency. (2) The trailing type, with its several branches spread on the soil, succeeds best in a hot sandy soil, indicating greater xerophytic tendencies. The Jumbo variety is an example. Now, the wild Brazilian species *A. pusilla*, is an erect plant, simulating the bunch variety and is reported as growing in dry woods and shady places. Another, *A. prostrata*, is more trailing and grows in open sandy places and so, simulating the prostrate cultivated variety, is

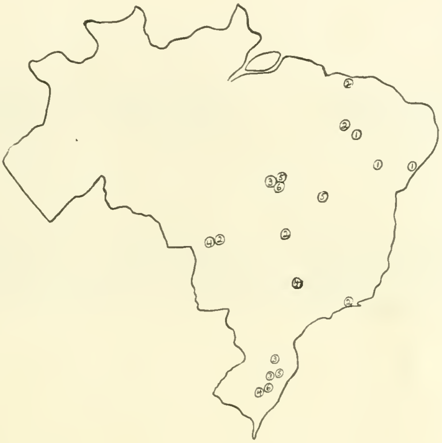

Figure 2
Outline Map of Brazil indicating the reported distribution of the different species of *Arachis*.

1. *A. pusilla.*
2. *A. prostrata.*
3. *A. villosa.*
4. *A. glabrata.*
5. *A. marginata.*
6. *A. tuberosa.*

more xerophytic. *Thus the possibility is suggested, first, that the cultivated bunch varieties are derived from such a species as A. pusilla and second, that the prostrate varieties are derived from A. prostrata.* Other evidence in support of this theory is seen in the marked difference in the histology of the fruits of the two domesticated varieties.

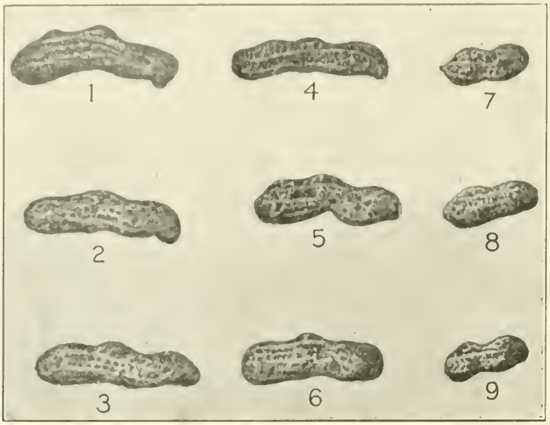

Figure 3 (after Dubard)
Types of peanuts taken at random from
1. Tombs at Ancon, Peru.
2. Java.
3. Tonkin.
4. Madagascar.
5. Madagascar.
6. Spain.
7. Dahomey.
8. Senegal.
9. Spain.

The following summary taken from Dubard further indicates the possibility of our present day varieties being derived from two such wild plants. He states that there were two types of peanuts distributed over the world from South America; one being a two-seeded Brazilian type and the other a three-seeded Peruvian type. The first form was carried to West Africa by Portuguese negroes, according to this author. It was taken to Europe by early travelers, the Portuguese being there the first propagators, as indicated by Parkinson and others. Further evidence in support of this eastward distribution, which seems more natural from the geographical standpoint, is, that (1) all early illustrations of European and Brazilian works show two seeds, and (2) those of West Africa are two-seeded. The Peruvian type was a variety created in Peru from a form carried there from Brazil some time before the sixteenth century. The question now arises as to the possibility of this plant being a different species from the common two-seeded type. Bentham states the number of seeds to vary from one to three in this genus. This being true, any species might well have been selected. However, Dubard later describes differences in the structure of these which are sufficiently marked to separate them into species. About Magellan's time, this three-seeded form was carried from Peru, to the Moluccas, Phillipines, Indo-China, Asia and Madagascar. If this is true, we should find the three-seeded fruits in these Pacific localities, and this Dubard proves to be true, by comparing specimens taken at hazard from Java, Indo-China and Madagascar. (Figure 3.)

Finally, if peanuts of to-day from Spain and North America be examined, the above two types will be found, indicating a meeting again of these, after having been carried around the world in opposite directions, yet remaining distinct in character. The Peruvian form was undoubtedly carried north and east, but at a date much later than its westward spread. The supposition, according to record, that the two were present at about the same time,—the one in Africa and Europe, the other in the Orient,—further supports this view. Watt states that one name given it in India, where it is much cultivated, is "Manilla-Kottai." This suggests its introduction there from the Phillipines. All these facts relating to the distribution are suggestive, when the lack of evidence of its presence during ancient times in China, India, Africa and Europe is considered.

Since Dubard does not describe the plants of his two forms, it is impossible to determine whether or not they might correspond to the erect and prostrate types. He does describe the fruit and seed of each, however, and it is found that his Brazilian form corresponds to some of the erect, and his Peruvian form to some of the prostrate varieties of to-day. If such a history is possible, which does not seem unlikely, the reported distribution of the two wild species—*A. prostrata* and *A. pusilla*—is again significant. The former, found in all parts of Brazil, would be the one most naturally carried to Peru rather than the more restricted latter species that is found only in the eastern part. The lack of full descriptive literature on the distribution and morphology of wild and cultivated varieties opens up opportunities for further investigation.

To-day, varieties are rapidly being increased in number by man. This fact, with the ease of intercourse between different countries, explains in part at least why there are such varieties as the Virginian, Spanish, African, Asiatic, etc. It does not indicate in any way the native home of the peanut, which is undoubtedly Brazil.

Recent Literature. During the last 25 years most of the literature on the peanut has been largely concerned with either its culture, uses or chemistry. The writer does not attempt to summarize these and includes titles of but a few of the more recent publications in his bibliography. By referring to the Experiment Station Record of the United States Department of Agriculture, many such references may be found. During the past few years, publications concerning its culture and varieties have been issued by several of the Agricultural Experiment Stations of those southern states in which the peanut is becoming an important crop.

Concerning the study of the plant from a morphological standpoint, little has been done. In 1895, Pettit[15] worked on the fruit stalk. She described its structure in detail and discussed its physiological relation to the plant.

Winton[16] published the results of a histological study of the mature fruit, undertaken especially to secure data for use in the microscopical examination of peanut products. In this relation he described and illustrated the cell structure of the fruit, testa and cotyledons. Adam[17] in 1908 published a fine work concerning its history, growth habits, varieties, culture, products and industry in western Africa.

General Morphology

Description. Since there are two well recognized forms of the peanut plant, the author suggests a division of the Linnaean species into the two sub-species—(1) *fastigiata* for the bunch type, and (2) *procumbens* for the prostrate type, and including under each a number of varieties. Adam gives the full species name *asiatica* for the former, and *africana* for the latter. Such are not only names of varieties and localities, but suggest an erroneous origin. The following is a description of the species, the suggested sub-species and the common varieties.

Arachis hypogaea

Family Leguminosae *Sub-family Hedysareae*

An herbaceous annual.

Roots—fibrous, delicate and white when young. Root hairs usually in rosettes at the base of the side roots—rarely with normal tip hairs. Nodules spherical; surface gray, interior pink; appearing when plants are 8–10 weeks old.

Hypocotyl—2–8 cm. long, sometimes slightly swollen at base during germination.

Stems—30–80 cm. long, erect, or prostrate, more or less hairy, tough, flexible, slightly quandrangular. Main stem usually branching early into cotyledonary branches.

Cotyledons—low epigeal, green, with short, thick petiole; remaining fleshy for two to three weeks, when they dry and drop off.

Leaves—sensitive to light, 8–12 cm. long, alternate, stipulate, pinnately compound. *Stipules* linear-lanceolate, erect, striate. *Petiole* straight, firm, with a single groove along the upper side; a pulvinus at its base. *Leaflets* 2–5 cm. long, four in two pairs, oblong to obovate; apex rotund and tipped by a tiny spine; veins pinnately arranged; under surface slightly hairy; each attached to the petiole by a short pulvinus which causes them to close together vertically in pairs at night.

Inflorescence—an axillary, usually three flowered, fascicled and reduced head.

Flowers—yellow, the larger more terminal ones usually sterile and adorning the plant for some time; the more axial numerous, basal ones usually fertile, smaller, more or less hidden, and born on short peduncles which elongate after fertilization. *Calyx* forming a long stalk-like tube with one narrow lobe as a lower lip, the upper broad and four-toothed. *Corolla* with a large yellow, orange-striped standard, two small wings, and a tiny, incurved, beaked keel. *Stamens* ten, monadelphous, versatile; five often with fertile anthers attached near their base; the alternate ones absent or short and fixed at their center. *Pistil* monocarpous; style long, slender; ovary small, at base of the long calyx tube, one celled with one to six ovules; after fertilization the floral envelopes drop away, and the ovary, now sharp-pointed and strengthened, is pushed by the rigid, recurving pedicel one to three inches into the soil; after penetration it begins to swell and ripen into a fruit.

Fruit—an indehiscent legume, oblong, reticulated, thick, coriaceous, beaked, swollen around the contained seeds, provided with absorptive hairs when nearing maturity. *Fruit stem* or gynophore reddish and slightly hairy above the soil—white and matted with absorbing hairs below.

Seeds—1–1 ½ cm. long; cotyledons thick, fleshy, oily; radicle short, straight. *Testa* thin, papery, membranous, varying from cream to pink to dark red color.

Sub-species—fastigiata Waldron

Main stem erect and branches all in an upward diagonal position, giving the plant a bushy appearance. Fertile flowers axially grouped near base of plant. Fruit clustered below the main stem. Seeds usually small and oblong.

Variety—White Spanish

Plant 20–30 cm. tall in average soils—foliage abundant and heavy. Pods small, adhering well to the plant, entirely filled by two seeds with pink to brownish testa. Very productive with high oil content.

Variety—Red Spanish

Similar to the White Spanish, except that the seed coats are red and the pods somewhat larger—less productive than the White Spanish.

Variety—Valencia

Plant 25–50 cm. tall. Pods long, medium thickness, clinging poorly to the plant, and containing two to four closely crowded seeds with red testas.

Variety—Tennessee Red

Plants similar to those of the Spanish varieties. Pods long, clinging to the plants and containing two to six seeds with dull red seed coats.

Sub-species—procumbens Waldron

Loosely branching, spreading plants (semi-erect in one variety); the stems often later ascending. Flowers and fruit considerably scattered along the prostrate stems. Seeds large, more or less pointed.

Variety—North Carolina, Florida Runner, African, Wilmington, etc.

Rank growing plants with dark green massive foliage. Pods not clinging well, medium sized, containing two, sometimes three, moderate-sized seeds with reddish testas.

Variety—Virginia Runner

Similar to the North Carolina, but with much larger pods and seeds.

Variety—Virginia Bunch

Plants semi-erect with light foliage, often appearing among plants in fields of the Runner type. Pods large, adhering well to the plant, bright, clean, with two, sometimes three, seeds covered by light brown coats.

Variety—Jumbo

The same as, or possibly sometimes a strain of, the Virginia Runner or Virginia Bunch varieties.

HISTOLOGY

Root

The internal structure of the root of *Arachis* is of the normal dicotyledonous type. The epidermal relation, however, of young roots and the production of root hairs is quite striking. It has been reported by Pettit and Richter[18] that no hairs are produced on the plant. The author, however, found them on all plants examined but usually in different position from the normally produced tip hairs. Although the tip hairs were found, they were rare and appeared only on young vigorous plants. Usually they are present in the form of rosettes on and at the base of newly formed side roots. As seen in Plate LXXIX, Fig. 4, those produced nearest the base are comparatively long, but are gradually reduced in length until none appear. Aside from the position relation, these rosette hairs are of the normal root-tip hair structure. The tip hairs when present are usually rather short and scattered, occurring on young delicate, usually few-branched, roots. No hairs of either type have been observed on the main root either during germination or later.

Young elongating roots of the plant which bear no root hairs often have their cuticle mucilaginized causing the soil particles to adhere as if hairs are present. These roots are often very white, delicate and semi-transparent. Placing a portion of one on a slide for examination under the microscope must be done with care, for the epidermal and outer cortex cells seem readily to fall apart like so many poorly cemented bricks becoming loosened. This is due, in part at least, to pressure from within, since, as noted by Pettit, the inner meristems develop much faster than the dermatogen. This is markedly evident on the primary root where the surface cells are continually peeling off in rows or patches. Sufficient protection, according to Pettit, is afforded by a cutinized outer wall being formed by the cells which become exposed. Pettit also states that the lateral roots act in a similar way, but the present writer did not find this to be the case with his plants. These roots are always normal in this regard, although very delicate. As they increase in age, their outer surface is supplied with a regular periderm, and it is only in the early stages of radicle growth that this peculiar habit is observed.

Stem

The stem is normal for dicotyledons. The epidermis is composed of a layer of small, thickly cuticularized cells interrupted by stomata in young stems and by a corky lenticel proliferation in old ones. Three-celled hairs are scattered along young stems. These are typical of many other Leguninosae, each hair having a long pointed terminal cell with two tiny, flattened basal cells. There are also small crystal cells arranged in groups of two to four, each containing one rectangular crystal. The cortex is six to eight layered and composed of much larger thin walled cells. At the outer extremity of the primary bundle areas of the vascular system are large patches of highly indurated hard bast. This, along with the quite extensive and considerably indurated xylem, give a tough and flexible character to the stem. The pith in young stems is composed of round extremely thin walled cells filled with starch. As the stem matures, however, the pith becomes more or less broken down resulting in a semi-hollow condition. Concerning the cambium, a very interesting relation is noted later as to its development in the fruit stalk (see page 314).

Leaf

The leaf structure of the peanut is most striking, especially when the xerophytic tendencies of the plant are considered. There are numerous average-sized stomata on both surfaces that are neither raised nor sunken. They average from fifteen to twenty per square millimeter, and each is surrounded by two unequal subsidiary cells. Both epidermal layers are also supplied with numerous specially formed crystal cells (Plate LXXIX, Fig. 5.) which, when the leaf is young, are small, each containing a single rounded crystal. Later, these cells become fused into what might be called an "epidermal vessel," irregular in shape and containing two to thirty crystals arranged in clusters or irregular rows. Solereder[19] notes the presence of these, but does not mention the later fusion into one. In attempting to determine the origin of these, a stem apex with a young bud attached was sectioned. It was found that in very young leaves all the epidermal cells were alike, and devoid of crystals. Immediately after the last cell division, however, some of the cells increased in size to form the normal epidermis, while others remained small to become the crystal-containing cells. In some of these it was noted that the small crystal seemed to be a part of the nucleus, possibly formed within and by it. It was in a pellicle-like projection which later separated away. In all such young cells the crystal was imbedded in a protoplasmic matrix which gradually became mucilaginized in old cells. The mesophyll of the leaf is composed of a two- to five-layered palisade tissue immediately below the upper epidermis and a single layer of water storage cells next to the lower. These two features are more typical of a xerophytic plant than is the presence of the above mentioned stomata. A loose, comparatively thin layer of spongy mesophyll separates the palisade and water storage layers. The petiole and lower epidermis of the leaflets bear the typical three celled hairs already mentioned as found on the stem.

Fruit

The anatomy and physiology of the fruit were carefully studied for the purpose of discovering, if possible, some facts concerning its hypogeal development. Among other members of the Leguminosae to share this peculiarity are *Amphicarpa monoica*, *Trifolium subterraneum*, species of *Voandzeia* and of the new African genus *Kerstingiella*. *Amphicarpa* bears two kinds of flowers, and accordingly two forms of fruit, only one of which develops underground. The flower which gives rise to this subterrenean fruit is formed and always remains underground. *Trifolium subterraneum* bears but one type of flower formed in heads. The peduncle, after flowering, lengthens and sinks into the soil carrying the head with it. The seeds do ripen above ground, however, and will germinate.

Differing from both of these, *Arachis* and the other two genera mentioned above have a nearly uniform type of flower, the ovary of which is pushed into the ground by a growth at its base. The elongating fruit stalk is called a "gynophore."

Anatomy of the Young Gynophore. Observations by the writer correspond to most of those of Pettit, whose article is chiefly concerned with the structure and development of this organ. Sections made longitudinally through young flower buds reveal a nearly sessile ovary usually containing two parietal ovules each, on a short funiculus (Plate LXXIX, Fig. 8). There are 11 to 13 bundles which extend through the base of the ovary to the tip, branching more or less in their course. Along the inner edge of each bundle are tannin pockets. After the egg is formed and fertilized, the reduced ovarian axis begins to elongate to form the gynophore. The ovary and embryo sac remain unchanged in this condition until the gynophore is mature. This will be further discussed under physiology. The later cytological study of the embryo was not attempted.

The meristematic tissue which gives rise to this growth is mostly situated just below, and around the base of the ovary. That below the ovary forms the pith, while that around the base forms the bundle tissue and outer cortex. A few dividing cells forming the latter were found well up around the ovarian cavity. The epidermis of the tip becomes sharp pointed and highly lignified in its outer walls. In Plate LXXIX, Fig. 9, is seen the area at one side of the tip where the style was formerly attached. This style is terminal in very young buds, but the later lateral position of the scar is prearranged for by a special development of a few large lignified epidermal cells at one side of its base. (Plate LXXIX, Fig. 8). These grow forward a little, and form the sharp point of the ovary.

Anatomy of the Mature Gynophore. While the structure of this fruit stalk corresponds to that of the stem of any herbaceous dicotyledon, its manner of development resembles that of ordinary roots. There are no lateral appendages, and so no nodes and internodes. There are two distinct divisions of this organ (1) the epigeal part, with smooth, red pigmented surface bearing a few three celled hairs similar to those on other parts of the plant; (2) the hypogeal white part, whose surface produces single-celled absorptive hairs. (Plate LXXIX, Fig. 7.) The surface of the aerial portion is covered with stomata and lenticels. The epidermal layer of this has a few scattered crystal cells similar to those found on the stem.

The cortex is composed of six to eight layers of round thin walled cells. Internal to this is the vascular system composed of a ring of bundles, each with a large patch of highly lignified bast on its outer side.

Concerning the cambium layer, Pettit says, "There is an indication of the formation of a cambium ring, although it never occurs even in the oldest portion of the organ." She goes on to explain the apparent presence of meristematic tissue between the bundles, which the writer considers interfascicular cambium. She continues, "The cells are, in their early stages, no larger than the pith cells, but as they become older they increase rapidly in both tangential and radial diameters. This process, however, appears insufficient to keep pace with the growing intrafascicular cambium, and they now become meristematic, forming new walls which are at first tangential; later radial walls are found. In this manner arise clusters or bands of relatively small cells extending from bundle to bundle. While these small cells appear like the ordinary meristematic tissue of stems whose cambium is formed after the bundles appear, they do not continue meristematic; at least in the organs studied there is little evidence that these small cells produce lasting tissue of any kind, and none whatever of the formation of phloem and xylem elements." The above description is correct for the cause and manner in which they develop and appear, but the writer feels that this tissue is in all respects meristematic as a part of a continuous cambium ring. The author has seen xylem and phloem elements cut off from it to form secondary bundles, and thus proving that it has the ability to form these. Also, the presence of such cells in a nearly continuous line with the intrafascicular cambium suggests an hereditary tendency to form it even though it is less active than in other stems. It does apparently connect the xylem patches, but so does the corresponding tissue of many stems. The fact that they divide even once is sufficient proof, since the resulting cells are permanent. The pith is composed of thin walled cells stored with starch until the fruit begins to form. Later the pith breaks down as does that of the stem and the gynophore becomes more or less hollow.

The anatomy of the subterranean part of the gynophore differs from that above ground in the following ways: (1) All of the epidermal cells become extended outward into long absorptive hairs simulating typical root hairs. (2) a growth in thickness occurs by a process similar to that of periderm formation, by which the diameter of the subterranean part is somewhat increased. Pettit notes another difference in the absence of what she calls *plasmolytic cells* situated in the center of the cortex of the epigeal part. These cells have not been observed by the author.

The absorptive hairs (Plate LXXIX, Fig. 7) are large, unbranched, one celled and average nearly a millimeter in length. Each is slightly enlarged at the base which represents the size of the original cell from which it springs. No stomata were seen, but lenticels were present which developed into a white proliferation of cells when exposed to a moist atmosphere as did some of those of the epigeal portion.

The examination of a cross section of this hypogeal area showed the outer layer of the cortex dividing into two to four layers of cork-like cells. The cells of this sub-epidermal layer are somewhat larger than those of the rest of the cortex. Although these apparent phellogen derived cells have tbe appearance of periderm, according to Pettit, they are free from suberin, as would be expected from the presence of absorption hairs on their exterior.

Anatomy of the Young Fruit. As noted above (see page 316) the ovary, situated at the tip of the gynophore remains inactive until the time comes for fruit maturation. The epidermis at this time is composed of much deeper and narrower cells radially, than that of the non-hairy part of the gynophore. They become deep, tapering and lignified at the tip forming a hard, but not capped apex. The lumen of those at the tip contain numerous granules that are not evident further back and suggest a relation to the geotropic reaction of the gynophore. Three or four hypodermal layers, that later form the outer mesocarp, are composed of markedly cylindric cells. A branching bundle system within this is a continuation of that of the gynophore which gradually disappears toward the tip. Just interior to this are a few layers which later assume marked appearance and importance as tissue which becomes gradually lignified to form the strengthening inner shell layer of the fruit. The innermost tissue next to the ovarian cavity is composed of several layers of tiny nearly square cells arranged in radiating rows.

Anatomy of the Developing Fruit. When the ovary begins to enlarge, the epidermal cells elongate longitudinally and later become ruptured. This epidermis with the subjacent layer, is rubbed or stripped off, which is a very unique event in fruit maturation. Apparently the epidermis does not keep up with the growth from within. Within the epidermis several layers of periderm have already appeared, similar to, and continuous with, that of the lower end of the fruit stalk (gynophore). Till this occurs and until the fruit is one half to three quarters full size, no hairs are formed on any part of the ovary or young fruit. About this time, however, and lasting until the fruit is mature, there appear on this layer irregular, often branched, one-celled absorptive hairs (Plate LXXIX, Fig. 6).

The developing bundles have increased in size, and with their connecting branches, form ridges which later give the reticulations to the fruit. Meantime a few layers, just interior to these, are becoming remarkably indurated to constitute the solid enclosing chamber of the mature fruit. The inner endocarp area of small cells has enormously thickened and from the time the fruit has begun to swell until nearly ripe, this area is composed of very large, thin walled, pith-like cells which contain sugar. This area remains thick and the developing seed small until a short time before maturity. It forms a large part of the fruit at this time.

Anatomy of the Mature Fruit. Winton notes the presence of an epidermis and states that it is not easily seen. As noted in the discussion of the developing fruit, this could only be a pseudo-epidermis that he has mistaken for the already shed epidermis, and the fact that it is the small-celled third layer of the ovarian tissue may explain why it is distinguished with difficulty. As in the nearly mature fruit, absorbing hairs are present as wall extensions of it. These hairs were not observed to be as often branched as in the younger fruit. One or two appeared to be septate.

Just below the outer absorbing layer are several rows of brick shaped, thin walled cells, simulating those of the hypogeal gynophore in appearance and position. Within this are several layers of thin walled cells that have collapsed. Apparently new cells were not produced for this area to allow for the expansion of the fruit. These surrounded a few layers of unbroken, rounded cells in which are embedded the branching vascular bundles. Large bundles are arranged longitudinally, and are connected by short smaller cross bundles, the whole system forming the reticulation of the peanut shell. The xylem and phloem of these have become highly lignified.

Attached to the inner edge of the bundle network is the solid hard enclosing shell of the peanut, composed of the now mature lignified mass of cells mentioned in connection with the maturing fruit. The cells forming this are now remarkable in their shape, size, wall thickenings, and branches. (See Winton's article.)

The structural relation of the carpellary wall to that of a leaf, from which it is modified, is recognized, but with more difficulty than that of many aerial leguminous fruits. By carefully splitting open a fruit, as one ordinarily shells a peanut, it will be noted that the seeds are attached to the somewhat convex side opposite that of the beak. Thus the dorsal is recognized from the ventral suture. Histologically these areas are not discernable in young ovaries, except by noting the location of the ovule attachment, or that of the style. This also locates exactly the position of the beak in the mature fruit. In mature fruit shells there is a ventral suture along which they quite readily split, due to a weaker, less lignified, loose line of tissue as seen under the microscope.

Concerning the exocarp, mesocarp and endocarp and their origin, there is such a marked change and fusion of parts that any sharp line of demarcation is impossible. The exocarp, which is typically derived from the lower leaf epidermis, is lost during fruit maturation. The name endocarp, derived from the lower epidermis, might be applied to the soft, internal tissue called inner parenchyma by Winton. Practically the whole of the shell then would be the mesocarp and can be subdivided into *hypoderm*, *bundle area* and *fibre layer*.

The anatomy and cytology of the embryo not having been attempted, that of the mature seed is also omitted. For details of the latter the reader is referred to Winton's article.

Physiology

Root Hairs

To the writer, the thought suggested by others, as noted in the histological discussion, that *Arachis* bore no root hairs, seemed contrary to expectation. The plant, with a semi-xerophytic tendency, and growing well in a warm, loose soil, would be expected to have them, at least when moisture is sufficient to stimulate their production.

Plants started in the greenhouse and carried on in flower pots were therefore examined. Of twenty-five plants, one showed hairs present near the tips of two of the young vigorous growing roots.

They were, however, very short and comparatively few in number. It was noted, however, with some surprise, that on the roots of nearly every plant, tufts or whorls of hairs were present as rosettes at the base of some of the side rootlets. These were much longer than the first-mentioned type, as is set forth in the figure. The possibility that the influence of potting may have in some way caused the development of these rosettes as well as tip hairs, led to an examination of the roots in the center of pots, and of roots on plants which had been carried forward in boxes (2 x 3 x 1½ ft.) Such roots have less air drainage than those along the inside wall of a flower pot. Plants were removed, their roots carefully washed out, and tufts of hairs were found at the base of many newly formed side roots.

The presence of normally produced tip hairs was carefully watched for, but none was found. The only plant mentioned which had these was one, the roots of which were in a moist air compartment, formed by the drainage hole of the bottom of the flower pot, the broken crocks just above, and the cinder bench below. This suggests, therefore, that optimum oxygenation is a necessary factor for the tip hair growth. None were found on the roots of any plants in the soil, either under dry or moist conditions. Since the plant seemed to have at least a hereditary tendency under certain conditions to produce these normal tip hairs it was considered worth while to determine, if possible, the exact causes or stimuli which affect their growth and that of the rosette type. Observations and experiments were, therefore, carried on with this in view, as well as to determine the causes and method of production of, and differences between, the two forms. The results of this investigation are described in succession to the following discussion of the works of others on this subject.

Concerning the presence and absence of root hairs, Strasburger[20] states that in some few instances as in some conifers plants bear no root hairs. Jost[21] says that few plants produce none, probably referring to aquatic types. Haberlandt[22] refers to two stages in the specialization of absorptive tissue in plants. (1) Some plants are content to increase their absorptive surface by a greater output of side roots, the epidermal cells of which are flat or slightly convex in their outer walls. But he states that this type includes marsh and aquatic plants, and is less advanced in this regard. If this is a stage in specialization, would it not be possible to think of it as a reduction change following a former evolved condition in root hair production due to adaptation to changed environment? Corn and many other plants produce none when put in water. (2) Other plants produce root hairs near the tips of their roots by elongation of the outer epidermal cell walls. Since many hair producing plants cease to bear these when in contact with water or saturated soil, it would seem to the writer that instead of being two stages of specialization, it is an example of two types of absorptive tissue dependent on ecological factors. It is a more or less epidermal surface extension for absorption, dependent upon the amount of causing stimuli present. The hairless aquatic plants may have had hairs at some time, and some do produce them when growing in dry soil again.

As to the cause for root hair production, Pfeffer[23] states that too little or too much water hinders, while darkness and contact accelerate. Snow[24] in an extensive investigation on the causes of their development finds that they are accelerated by a retardation of growth, by mechanical means, or substratum resistance, especially if the roots of such are allowed to grow in a moist atmosphere. She finds that they are retarded by a saturated atmosphere at high temperatures, by a lack of oxygen, and by a saturated soil; light and darkness, however, have no material effect.

Observations and Experiments with Root Hairs

It was noted that if seeds were planted in a heavy soil and germination was retarded by lack of moisture, the hypocotyl would sometimes swell considerably and give off adventitious roots which branched profusely. By drawing the soil away, after the radicle had grown an inch or two, until the lower end of the hypocotyl was well exposed, the upper part of the side, and of the adventitious roots with their hairs could be kept growing in saturated air. This gave a good opportunity to determine the effect of sunlight on the growth of hairs, as compared with those on a few plants whose roots were kept in the dark, but also exposed to the air. The foliage of both sets of plants had the same leaf exposure, so that the activity and growth were as near the same in all as possible. In all cases, both in light and in darkness, the rosette hairs were found at the base of the side rootlets and there was no marked difference in their size or abundance. This corresponds to the results of Snow, except that in her observations she noted a slightly longer growth in the dark. This was possibly due to a greater drying out in light in her experiments. No normally produced tip hairs were observed.

Temperature. Plants with roots exposed were kept at 90° F. to 100° F. in a moist chamber. Others were kept at 60° F. to 70° F., the other conditions being the same. Rosette hairs appeared on all the plants, but at the higher temperature they were much more luxuriant and abundant. Tip hairs were found near the tips of several roots on two of the plants growing at the high temperature. These two plants producing both types of hairs were the most vigorous of the set of twenty in the experiment. These results do not correspond with those of Snow, where a high temperature and humidity retarded their growth. This can possibly be explained by the fact that the peanut requires a higher temperature for optimum growth than those plants with which she experimented.

Soil. Plants in loose sandy soil composed of one-half light loam and one-half sand grew vigorously. After germination they produced numerous long and almost pure white roots, a few of which, after reaching the side of the pot or box, bore a limited number of tip hairs. No rosette hairs appeared until the plants were one to two weeks old and quite well established. Tip hairs appeared on one plant only, of those that were older than three weeks. Plants in light loam, without any mixture of sand, grew slowly and the rosette hairs were the first and only type observed, after the side roots had developed. They appeared on all the plants examined after from one to two weeks' growth. Tip hairs were observed on a two months' old plant which had been retarded in its growth, and, when repotted, a few delicate roots appeared which bore a very few scattered tip hairs.* Rosette hairs could be found on any plant of any age, except the young vigorous specimens of less than two or three weeks' growth.

On seedlings of various ages no hairs of either sort were ever observed on the primary root. Many seeds germinated in sandy soil, and on sterilized wet cotton in test tubes, did not produce them even under optimum conditions of heat, air and moisture. With sufficient moisture, however, rosettes always appeared on the bases of the side roots. Those in test tubes grew slowly because of lack of water, and as a result rosette hairs were the only type observed. These were absent on the roots furthest from the moist cotton.

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*Of about fifteen such plants examined this one was the only one in which they were observed.

Concerning the function of root hairs on the radicles of seedlings, Haberlandt and others state that one reason for their early formation on these, is, that the main root may have sufficient anchorage in order to rapidly penetrate the soil for its immediate needs. Evidence presented by the peanut thus entirely contradicts such a view, indicating that they are not necessary for this purpose. Such seeds as those of the peanut can alone supply adequate food material for germination if water is present. The radicle elongates and penetrates a light soil with great rapidity without them. This may be one reason why a light sandy soil is best for this species. This thought also suggests an interesting relation to the hypogeal fruit production. The plant always maturing its seed under ground would not need such anchorage for its first root growth, even though it were a desirable feature. The fruit wall acts also as an aid. The writer feels, however, that the reason why hairs are not present here is that the outer layers, in stripping off (see page 314), do not allow their development. The fact that side roots, which do not show this peeling, will form them under proper stimuli is evidence of this. Even these do not often produce them, probably because of their very loose structure (see page 314). The cells which are best adapted to respond are at the base of side roots, thus rosettes are formed.

To summarize these observations, the results of the root hair experiments on the peanut indicate that (1) light and darkness have no effect on their production; (2) high temperature, with sufficient moisture and air, accelerate the growth and production of the rosette type; (3) loose sandy soil, with root aeration, stimulates hair growth on the tips of young plants, and possibly also on old plants, if a period of retardation is followed by a suddenly renewed root vigor. The rosettes of hairs may appear on any plant of more than one to two weeks' growth. The tip hairs are found only on young roots that are undergoing a vigorous elongation in a natural or artificial moist air space at a high temperature. Low temperature, lack of oxygen and wet heavy soil prevent the normal tip type from appearing and retard the rosette form. None appear on the radicle. The cause for the production of the rosette hairs at and on the base of the side roots is hard to explain. It may be that, at the time the side root is penetrating the cortex and epidermis of the main root, the root tip tissue here is more active and vitalized than later, and so is more sensitive to external stimuli. It was noted that when tip hairs were produced usually no rosettes were present. Possibly these offset the need for tip hairs, or at least utilize energy which is lacking for their later formation.

Absorption

From the foregong observations on the roots and root hairs of the peanut, it is evident that the young plant absorbs its water and mineral foods by any one, two, or all of the following means, depending on environment and growth conditions: (1) Through the epidermis of young roots the thin cuticle of which is mucilaginized; (2) By means of normal tip hairs on vigorous growing roots; (3) By means of the rosetted, basal hairs. The first and last are undoubtedly the most important of these. As soon as the fruit stalks appear and reach the soil, hairs are at once formed from the epidermal cells of these. The older plants then have a much more extensive absorbing surface from the formation of a considerable number of gynophores.

Proof that these hairs do supply water to the plant is indicated by the fact that, according to Pettit, when the roots of such are severed the plant continues active and apparently uninjured for some time. The xylem of the gynophore bundles, although not very large, is sufficient to carry a considerable quantity of material. The nearly mature fruit must also absorb some water as indicated by the presence of delicate absorbing hairs. How important this is it is difficult to say. That they are not absolutely essential is evident from the fact that the fruit continues to swell somewhat if transferred from the soil to the air, causing the hairs to dry up. The absorbing fruit stalk undoubtedly takes the place of roots to a certain extent. Root tubercles, which also appear at about the same time, should be noted too in this relation, since some other nodule-producing members of the Leguminosae, as is well known, seem to have a reduction of root hairs. The absorbing surface of roots alone on these older plants is comparatively small.

Development

Germination. The writer, in attempting to raise plants for study in the greenhouse, had some difficulty in keeping insects and mice away. Some of these seemed to have no difficulty in locating the seed even before germination. A wire cage was finally built which controlled these pests. Further trouble was experienced, however, unless great care was used in planting and watering. It was found that unless they were brought forward in loose material like sphagnum or well aerated sandy soil, they rotted in one to two days. If pure sand were used, they would rot if kept even moderately wet. The method finally used which succeeded was to plant several together, allowing a considerable degree of aeration. When these produced an inch or two of radicle, they were separated and transferred to individual pots with the cotyledons half exposed. Seeds planted in the shell succeeded well, as this seemed to allow also for free aeration. The ease with which the seeds rot is likely due to its weak protection by the testa. This thin papery coat is easily ruptured and the embryo, rich in food material, seems to be very susceptible to infection by molds and decay-bacteria. If growth is rapid, however, the increased oxidation gives it vitality to resist. Those who raise peanuts say that good drainage in a loose soil is absolutely essential for success. The plant must start quickly and be kept growing. If planted in the uninjured shell, which is sterile within, there is less likelihood of infection before it gets well under way. A comparison was made by Bennett[25] of growing peanuts from shelled nuts, nuts broken into two parts, dry and unshelled nuts, and unshelled nuts which had been soaked in water for 12 hours and buried in the earth below the frost line for different periods. The most perfect stand was obtained from nuts planted in broken pods. The results seemed to indicate that when nuts had been thoroughly wet and moist for a short time they would produce a good stand, and save the expense of shelling. This corroborates the author's thought that the seed benefits by free oxygen and protection furnished by the shell in order to start its growth successfully, at least in anything but an extremely loose soil.

Later Growth. After the radicle has reached two or three inches the cotyledons are pushed about 2 cm. into the air by the elongating hypocotyl. The hypocotyl often becomes thick and fleshy in its cortex. This is more marked when growth is retarded from some cause, and then the lower end becomes tuberous from a deposition of sugar. The roots of such are not able to utilize the food as fast as it is supplied from the seed. When the soil is light and the temperature optimum, the formation of an extensive root system results (see page 323). Under these conditions while the food of the cotyledons is still available, the plant grows rapidly for about two weeks, followed by a period of very moderate development. Audouard[26] states that the plant grows slowly during the first half of its existence, and that the most rapid growth takes place after about ten weeks. This indicates a relation to the formation of the gynophore and root tubercles again, both of which appear later. This makes possible a greater activity in growth of the plant. One feature somewhat difficult to understand is the presence of the numerous stomata on both epidermal layers. How is the water balance kept with such a reduced hair surface, especially on the roots of plants not yet producing fruit?

If the seed is deep, the hypocotyl elongates accordingly. If the seed is planted in the shell, it will push up through three or four inches of soil. It is often much curved and twisted in its efforts to extricate the cotyledons from the shell. These remain green for two or three weeks, when they wither and drop. Concerning the presence of food materials during growth Audouard states (1) that there is sugar in all parts of the plant, which decreases in amount during fruit maturation; (2) That starch in the root and stem increases from the beginning to the end of vegetation; (3) that fats increase for six to nine weeks, that is, until the fruiting period, when they suddenly decrease in the vegetative organs; (4) that proteins decrease in roots and stems at flowering time and increase in the fruit. The writer has observed that the gynophore is well stored with starch until the ovary begins to grow, when apparently much of it is carried as sugar to the inner fruit tissue, forming there the broad, delicate-walled sugary endocarp (see page 319),—thus the reason why immature fruits are sweeter. Some of this sugar at least is apparently gradually transferred to the testa and there stored temporarily as starch. Later, both this and that from other sources (gynophore and stem) are transferred to the cotyledons and largely stored as oil. Since the carbohydrates are early furnished and carried to the gynophore and stem, the thought arises as to the possibility of the fruit maturation being largely independent of the roots of the plant. The other food materials can be obtained from the soil by the fruit, and proteins can be formed in darkness, so there is no known reason why this should not occur.

Biological Considerations Concerning the Fruit and Gynophore.

Observations on the Gynophore. In his work on "The Movements of Plants," Darwin[27] says that while apheliotropism may act in some slight measure on the downward growth of this organ, geotropism is unquestionably the exciting cause. The writer proved this by inverting two plants which had produced several gynophores whose tips were about to pierce the soil. As seen in Fig. 11 the tips turned away and became reversed in position. This not only proved the effect of gravity, but also that the hydrotropic reaction of the organ was weak or lacking. They acted in a similar way even if the soil was saturated. When tips were allowed to penetrate wet sphagnum, and then the plants reversed, they would recurve downward and grow out of it. When the plants were righted again the tips also turned back thus forming an S curve (Plate LXXX, Figs. 11 and 12). The presence of definite granules in the lumen of each of the epidermal cells of the gynophore (see page 318) at the tip, and their absence anywhere else, suggests the possibility of such being the structures by which this organ perceives when it is out of line with gravity. This has been discussed by others in connection with the presence of starch grains in root tips. The writer found that by cutting off the tip of the gynophore, growth continued, but there was no reaction to gravity when the plant was inverted. The apparent homology between the behavior of the root apex and of the gynophore apex is highly suggestive.

Darwin refers to the means by which this organ penetrates through the soil. He says, "the sharp smooth point of the gynophore enables it to penetrate the ground by mere force of growth, but its action is aided by a circumnutating movement." The anatomy of the organ is also suggestive. The patches of hard bast give strength, while their separation, even though in a close ring, gives pliability. Pettit states that the hairs produced at the tip are also an aid in holding it firmly. Although this happens to be of some assistance, the writer would question to what extent, since the relation of the radicle to soil penetration puts a new light on this matter. Hair experiments, similar to those of Pettit, were made with plants bearing young gynophores, which had not yet reached the soil. Pettit found that by putting these in a moist chamber a narrow zone, averaging 3 mm. in length, always appears one to eight millimeters from the tip. The writer found by repeated experiments that this zone might be as much as 5 cm in length (Plate LXXX, Fig. 13).

In discussing these in relation to those of roots, Pettit says: "In comparing the growth of gynophore hairs with that of root hairs it must be remembered that the growing point of the gynophore corresponding to the punctum vegetationis of the root lies just below the ovary which occupies the extreme tip of this organ. The ovary, however, is almost microscopically small and remains so during the growth of the gynophore. To illustrate the extremely small space occupied by it, the hairs which were not more than one millimeter from the tips of the gynophores as mentioned above were still below the growing point under the ovary. While this difference in the position of the growing point exists between root and gynophore, the difference which it makes in estimating the relative distances of the hairs from the tips is practically nothing.

"The resemblance between these hairs and those of roots was further tested by repeated experiments in pulling young gynophores carefully from the soil. The minute portions of earth clung to the hairs and refused to be separated from them in the same manner as in the case of root hairs. In several instances these hairs were tested for acids and were found to respond readily to the litmus paper test.

"Still another experiment was made which furnishes strong evidence that one function of the gynophore hairs corresponds to the chief function of those of the root. A large, well developed, thriftily growing plant was cut in such a manner as to separate the whole root system from the stems, but the latter were still connected with the ground by numerous well grown gynophores. The result was that the plant so treated after two weeks still presented nothing to a superficial inspection to distinguish it from others in its vicinity whose roots were left intact. Closer examination showed that some branches were dead; but the majority were putting out new leaves which appeared quite as strong and healthy as any of those on similar plants in the vicinity which were supported by roots. Unfortunately these experiments were begun late in the season, and the appearance of the frost prevented their continuance."

Fruit Maturation. Other writers state that all attempts to make the gynophore produce aerial fruits by digging away the soil as the gynophore elongates fail. It is also well known that any such stem dries up unless it reaches the soil by the time it is two to three inches long. The length to which it grows before drying varies with the humidity of the air. Experiments were thus made in an attempt to determine what caused the ovary to enlarge, and what would prevent it. Plants with gynophores of various lengths were put in a saturated atmosphere and not allowed to penetrate any substratum. In all cases the gynophores continued to elongate and become green for about a month. They usually attained five or six inches in length and then wilted just back of the ovary. The longest gynophore produced in this way was seven and one half inches in length—nearly twice as long as any seen by the writer in the soil. Hairs developed which gradually died away until there were but a few near the tip. Other gynophores were allowed to grow in test tubes of tap water, some kept in the dark, others in the light. These produced no hairs. Two of those in darkness, after eight weeks, produced a small one seeded fruit. The remainder produced none. Others were allowed to grow into sphagnum and pure sand and resulted in fruit formation in both cases. Gynophores which had penetrated soil and whose ovary had begun to swell were exposed to a saturated atmosphere and to ordinary greenhouse conditions. In the former case, the fruit turned green and continued to grow slightly, while the latter turned green and remained small.

The results of these trials, although not at all conclusive as to evidence offered, indicate that (1) a *thigmotropic*, *hydrotropic* or *apoheliotropic* stimulus, or a combination of these, is necessary for the ovary to begin maturation, but (2) that a continuation of such is not necessary, since the ovary continues to develop somewhat if removed from the soil after its growth has begun. This development, however, is somewhat abnormal. All successful experiments were produced in complete or partial darkness, although those in the moist dark chambers failed. This suggests the necessity of the first two factors, i. e., water and contact. More research is necessary in order to clinch this point and determine which of the three is the most important.

From the writer's observations and from the varying results of the above attempts at fruit production, he feels that two things should be kept in mind—(1) the condition and activity of plant growth, (2) the ability of the plant to possibly supply the necessary substances to the fruit in two ways—(a) by direct absorption of some of them from the soil with the carbohydrate supply from the plant; (b) by transfer of all necessary materials from the vegetative organs into the fruit. A number of plants that became pot-bound, when several gynophores were being formed on them produced only one or two small fruits. Similar plants that had abundant pot-room produced several. Weak plants may have been experimented with. In the field, many poor fruits without seeds called "pops" are found—due possibly in part to this same cause.

The chlorophyll formed in the fruits exposed to light was found to be exterior to and around the bundles that form the pericarp reticulations. The testa and cotyledons also became green. This is an interesting point since it indicates the retention through long millenia of the factors necessary for chlorophyll formation.

Watt has observed in India that red ants are frequently found working harmlessly around growing fruits in the soil. This would be of benefit to the fruit since aeration of the surrounding soil would allow for greater absorptive hair development. What benefit the ants might obtain is hard to tell.

Fruit Hairs. It should be kept in mind that the fruit hairs are different in origin from any found elsewhere on the plant (see page 319). Points of evidence indicating this are—(1) that the epidermal and subjacent layer of cells is seen to be thrown off (see page 319 and Fig. 6). (2) That the hairs are different from any found elsewhere on the plant, all of which are truly epidermal. (3) That this difference, that is the bifurcations of the hairs, indicates the irregularity of growth of the mesophyll of leaves. (4) That no hairs appear on the fruit until it is well grown and the two outer layers have been discarded.

Other queries raised here are: why is the second layer of cells discarded as well as the epidermis? and why doesn't the periderm-like tissue begin its formation by divisions taking place in this layer, as is the case with the hypogeal gynophore, instead of in a deeper layer? One possible answer is, that this corresponds to that layer of water storage cells next to the lower epidermis of the leaves of *Arachis*. This layer still persists in the carpel, and, being large-celled and less able to divide, is thrown off with the epidermis.

Conclusions from Physiological Studies

It remains to be considered how far the facts ascertained in this study contribute to the knowledge of hypogeal fruit production. The fact that the fruit of so many plants of varied families seeks the ground must be regarded as significant. Tschirch,[28] in a paper on Leguminosae, says that one group of nitrogenous compounds produced by the Leguminosae can be formed only in darkness, and suggests this reason for such a habit. It has also been suggested that the fruit is thus protected from animals.

Concerning the present studies, the following new facts stand out quite prominently—(1) The tendency to fruit formation in moisture and darkness. (2) The formation of periderm-like tissue on the hypogeal gynophore and fruit. (3) The formation of hairs on the gynophore-epidermis and pseudoepidermis of the fruit. That water is absorbed by these hairs is undoubtedly true. The most puzzling new structural feature is the second, where thin-walled cells, not suberized, are laid down by a late-formed cambium layer. Such is rare in leaf tissue. The bud scales of Horse Chestnut and other trees produce a limited amount of suberized tissue in this way. Two to three layers are always produced on the gynophore when this organ is subjected to a saturated atmosphere. This suggests a possible water storage tissue, or it may be a result of pressure from within when more water is absorbed. Cells are possibly necessarily cut off to allow for this expansion.

Finally, it is suggested that if the plant does not require more nourishment from the soil than might be supplied by root hairs, and yet forms such hairs on the gynophore and nearly mature fruit, it may be more advantageous to take some of its food by this special method. Perhaps certain desirable changes are made possible by such foods always being in darkness. Is it then darkness, or extra water supply that the fruit seeks? Whatever the reason, the resulting advantage is full maturation and selective survival of the seed, which is highly concentrated in its food constituents.

Uses of the Peanut

To most people outside the peanut growing sections of the country, the peanut suggests only an unessential food article,—a delicacy to many,—in the form of the roasted or salted nut, peanut confectionery or peanut butter. During recent years, however, and especially in the past year or two it has become of utmost importance as a staple article of diet and otherwise. In the cotton growing states it is saving the day for many farmers who have failed with cotton growing because of the new insect pests or other reasons.

Uses as Human Food. The following from Beattie[29] in this connection is worth quoting: "The use of the peanut for eating from the shell is most important and popular, but the quantity of shelled peas that are first roasted and salted and sold by the pound is constantly increasing. Some of the better grades are first shelled, then roasted after which the halves are broken apart and the germ removed giving the meats a blanched appearance rendering them very desirable for table use. Great quantities of shelled peas are used every year in the manufacture of peanut candies and brittle, both alone and in combination with other nuts, pop corn or puffed rice."

During recent years great quantities of shelled peanuts, especially of the Spanish variety, have been employed for the manufacture of peanut butter. It is used in the preparation of vegetarian meats after a portion of the oil has been pressed from the nuts. This extra oil and that pressed from nuts grown for the purpose is used in thinning peanut butter and is as good for every purpose as is that of the olive. It is one of the sweetest vegetable oils. Articles fried in it keep well for a longer time than in olive oil and have an agreeable odor and flavor. It is mixed with cotton-seed oil to improve it for salad purposes.

Peanut meal or flour of finely ground nuts is used in confections, cakes and bread making. It is used as a substitute for rice and other flours. Watt reports that in India the unripe nuts are sweeter (as indicated in the author's discussion on the physiology of the fruit) and, being more easily digested, are given women whose milk supply is insufficient for their children. These unripe fruits, when fresh, make an agreeable boiled dish. The very tender leaves of the plant are sometimes cooked with ground coconut.

Concerning the food value and change of the peanut from the category of a luxury to that of a more staple item of diet for man, the following is taken from "The Literary Digest" for April 13, 1918. "The peanut enters into the preparation of most of the vegetable 'meat substitutes' long warmly advocated by the vegetarians and now made more conspicuous by the governmental admonition to 'eat less meat;' and peanut 'butters' or 'pastes' are widely used. Today the value of the peanut crop, which is divided between the production of the promising peanut-oil, peanut-cake for animal fodder and roasted peanuts for human food, has begun to total many millions of dollars. At the University of Wisconsin, Daniels and Loughlin have demonstrated by feeding-experiments on animals that the peanut can supply adequate protein . . . in sufficient proportions for growth and reproduction. It can also furnish an abundance of the water-soluble vitamin. The food as used in the human dietary does not, however, yield the growth-promoting fat-soluble vitamin, which has come to be recognized as a remarkable constituent of butter fat and egg fat; nor are the inorganic constituents adequate in quality to supply sufficient calcium and certain elements. Of course, the peanut is not used as a sole source of nutrients for man; nevertheless, the delineation of its physiologic value enables one to define more intelligently the place which it can take in the ration. Daniels and Loughlin foresee an increasing usefulness for the peanut, now that its real value has been scientifically established. When we consider the broad areas, they say, which may be adapted for growing the crop, and the fact that our food supply tends toward a wider use of the seeds of plants, it seems appropriate to expect that the peanut, when rightly supplemented, will form a staple article of the human dietary. Like the soy-bean, which has lately come into prominence in American homes, the peanut needs only to have added suitable inorganic salts and the fat-soluble accessory to make it a complete food."

Uses as Food for Live-Stock. Beattie is quoted in this connection as follows: "In the factories where peanuts are cleaned, shelled, and graded for the market there is always a certain percentage of cleanings and inferior stock that can readily be turned into stock foods. The outside shell, or hull, of the peanut, is rich in food materials, but is extremely difficult to reduce to a condition in which it can be fed. In large cleaning factories the shells are generally used as fuel, and the ash resulting therefrom is valuable as a fertilizer. often containing as high as 3 per cent of phosphoric acid, 9 per cent of potash and 6 per cent of lime.

"The thin brown covering of the peas has a feeding value almost equal to that of wheat bran. These hulls are especially desirable for mixing with the smaller particles of broken peas for stock feeding. In large factories where peanuts are prepared for the manufacture of peanut butter and similar preparations the waste in the form of small particles of the meats and the germs is considerable and this is sold to farmers for feeding purposes. In some cases the waste is mixed with a portion of the hulls and finely ground or chopped before leaving the factory. Peanut hulls make an excellent bedding for use in stables, and by using them in this manner and hauling the manure upon the land their full value can be obtained.

"Broken peas and germs are used largely as a food for hogs, but both should be fed in moderation and in combination with some grain, as the peanut fed by itself will produce a hog having soft fat and inferior meat. The famous Smithfield hams and bacon come from hogs that are fed partly on peanuts, the practice being to turn the hogs into the peanut fields after the crop has been gathered and allow them to glean the pods that were lost in harvesting. The principal objection to the use of peanut by-products as stock feed is their tendency to become rancid very quickly. The germs, which are high in nitrogen content, become rancid and bitter in a short while and should not be kept on hand for a greater period than fifty or sixty days."

Peanut cake is a stock feed composed of the remains of seeds when expressed for oil and is extremely rich. As hay, peanut tops are worth just as much as alfalfa, pound for pound. Even the entire plant is used, and often chopped fine for this purpose. It forms a well balanced ration for dairy cows.

Use as a Soil Renovator. Here again the peanut is rapidly becoming a crop of much importance. Peanuts are valuable as a substitute for cowpeas, especially in certain soils that are not adapted to the growing of the cowpea. In many sections where the clovers and other soil-renovating crops will not withstand the heat and drought of the summer months the peanut will thrive and make an excellent growth. A crop of peanuts for forage can often be grown after the removal of oats or some other spring crop, and although they may be badly overgrown by crab-grass, the tops may be mown with the grass for hay, and the hogs turned in to root out the peas.

Miscellaneous Uses. The oil, beside its use as a food, is valuable in soap making, in lubrication and for illumination in some countries. The shell is often ground into a fine powder for polishing tin plate. It is said that tin plate manufacturers cannot get enough since this and middlings are the only two things that will put that mirror-like polish on tinware and not leave a scratch on the surface.

Summary of Results

The results of these investigations concerning the histology and physiology of *Arachis* present marked features which are summarized as follows:

1. It was found that root hairs were present on the plant, although reported as absent by two previous workers. These were usually arranged in rosettes at and on the base of side roots. Their growth is stimulated by a high temperature and humidity. The normally produced tip hairs appeared on very young plants whose roots grew rapidly and were exposed to moist air conditions. Later they never formed unless the plant showed a sudden renewal of growth vigor. Saturated and heavy soil conditions retarded the growth of the rosette type and inhibited the appearance of the tip hairs.

2. The hypocotyl shows a tendency to enlarge and become tuberous unless growth conditions are ideal. This is apparently due to a deposition of sugar from the stored food of the cotyledon which is unable to be cared for by the root as fast as it is supplied.

3. The stem is quite normal. Its epidermis, however, has crystal cells in groups of two to four. The pith breaks down causing the stem to become more or less hollow.

4. The leaf, with numerous stomata on both the upper and lower surfaces, has also, in both epidermal layers, small cells with a single contained crystal in each when young. Later these cells become fused into an "epidermal vessel," containing two to thirty crystals arranged in irregular rows or groups.

5. The fruit stalks or gynophores have been shown to be geotropic in reaction, and the epidermal cells of the carpellary tips are markedly granular, suggesting a possible perceptive relation in this regard. These organs are very weakly hydrotropic and do not react to light or darkness. The epidermis of the epigeal portion has crystal cells like those of the stem. The epidermis of the hypogeal part becomes elongated in the cell walls to form absorptive hairs. The second layer becomes cambioid forming a phellogen-like hypodermis, and this, by cambioid activity, may divide into two or three layers. The bundles are separate and highly lignified in the outer phloem and in the xylem. This gives mechanical strength for soil penetration.

6. The young fruit, as it begins to swell, bursts the epidermal and subjacent layers of the ovary, throwing them off. The next or third layer, now the pseudo-epidermis, forms irregular, more or less branching absorptive pseudo-hairs. These are different in nature from any of the other hairs formed on the plant, and are indicative of the irregular growth of the spongy mesophyll of leaves. Beneath this layer are developed several zones of cells similar to, and continuous with, the pseudo-periderm of the gynophore. This is a marked peculiarity in leaf tissue formation.

7. Attempts to produce peanuts in the air by various means failed to give definite results. Two succeeded by allowing the gynophore to grow into water; several when grown on sphagnum and also pure sand. None succeeded where the ovary was exposed to light. The results indicate that water is an important factor, but that contact, or darkness, or both may also be necessary. Young fruits, if previously in contact with soil, and then exposed to the air, continued to develop to a certain extent and turned green. The

re-formation and presence of chlorophyll in these indicate the retention through long periods of time, of factors for its formation in such a leaf structure.

8. The possible benefits derived from underground fruit formation may be (a) protection from grazing animals, (b) formation of certain proteins possible only in darkness, (c) more rapid and greater development in fruit size and number.

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EXPLANATION OF PLATES

Plate LXXIX.

Fig. 4. Root hairs (rosette type) as they appear on and at the base of side roots.

Fig. 5. A portion of the upper epidermis of a leaf showing numerous stomata and crystals in "epidermal vessels."

Fig. 6. A section through the outer layers of a peanut fruit which was about three-fourths grown.
  a—A portion of the epidermis and subjacent layer of the ovary still adhering.
  b—More or less branched fruit hairs developing from the former ovarian third layer, now exposed.
  c—Hypodermal tissue.

Fig. 7. A section through the outer layers of the hypogeal part of a mature gynophore showing:
  a—Hairs formed from the epidermal layers.
  b—Two layers of the periderm-like tissue (hypoderm).

Fig. 8. A longitudinal section through a young ovary just before the blossoming.
  a—Base of style.
  b—Pollen chamber.
  c—Enlarged and indurated cells which become the apex in Fig. 9.
  d—Ovules in ovarian cavity.
  e—Fibro-vascular bundles.

Fig. 9. A longitudinal section through a gynophore tip, the ovary of which is three weeks older than that of Fig. 5, and drawn to the same scale. Note the very slight difference in size, the style scar at *a* now pushed to one side by the enlarged epidermal tip cells which are shown at *c* in Fig. 8.

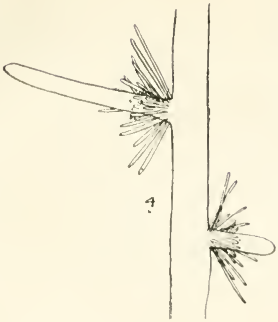
Plate LXXX.

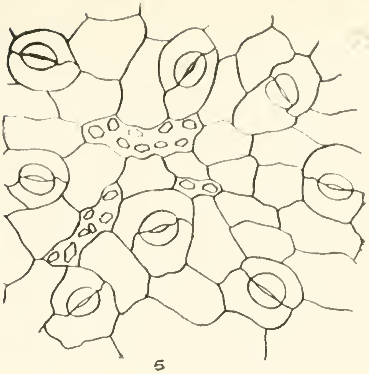
Fig. 10. Photo of a Spanish variety peanut plant showing flowers, flower buds and gynophores clustered together at its base as is typical of the *fastigiata* subspecies.

Fig. 11. Photo of an inverted plant showing gynophores recurving in reaction to gravity.

Fig. 12. Photo of plant which was righted again, shown in Fig. 11. Note the change of the gynophore tips again.

Fig. 13. Photo of a plant which, after forming gynophores, was put into a moist chamber. Note the profuse development of hairs resulting.





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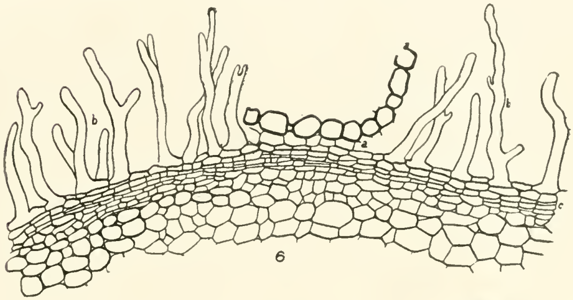

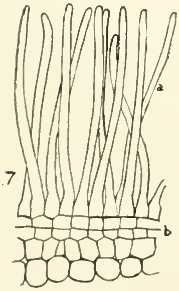

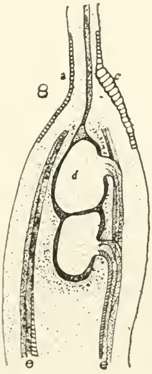

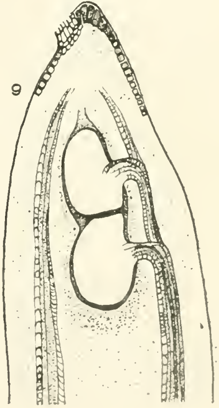

Waldron on Peanut

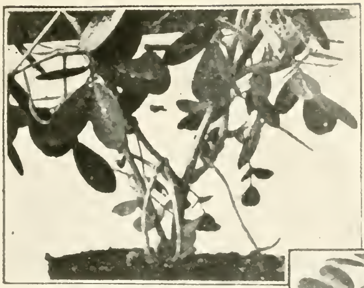

Fig. 11

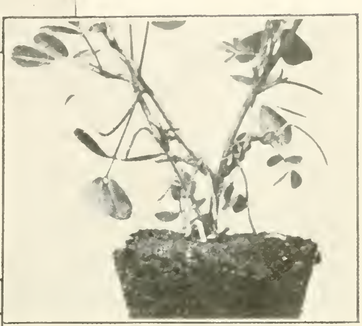

Fig. 12

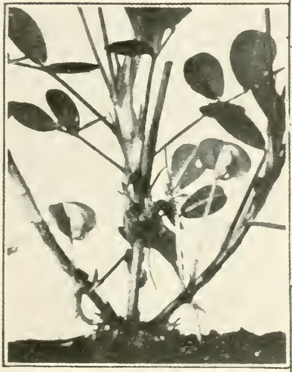

Fig 10

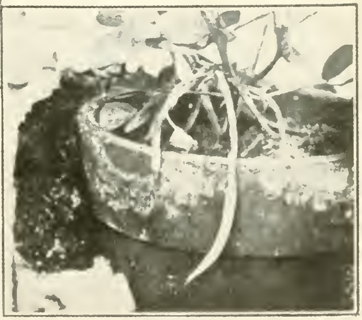

Fig. 13

WALDRON ON PEANUT

THE COMPARATIVE MORPHOLOGY, TAXONOMY AND DISTRIBUTION OF THE MYRICACEAE OF THE EASTERN UNITED STATES

BY

Heber Wilkinson Youngken, A.M., M.S., Ph.D.

With Plates LXXXI-XC

(Thesis presented to the Faculty of the Graduate School
of the University of Pennsylvania, May 8, 1915,
in partial fulfilment of the requirements
for the degree of Doctor of Philosophy).

I. Introduction

The writer, during the past years of his activities as a botanical teacher, examined minutely numerous drug plants along lines already mapped out by Tschirch, Oesterle and other leading pharmacognosists. In continuation of these studies and upon the kind suggestion of Professor John M. Macfarlane, of the University of Pennsylvania, he undertook the investigation of those species of the Myricaceae or Bayberry Family that are indigenous to the eastern United States.

This work was started during the winter of 1912 and has been carried on continuously up to the present date. During this period extensive studies have been made by the writer in the field, in the laboratory and in the herbarium of the University of Pennsylvania, also in the Academy of Natural Sciences of Philadelphia. The results already secured are such that the writer trusts to gradually undertake a monographic account of the family, which presents many features of great botanical interest.

The writer desires to express his grateful appreciation to Professor Macfarlane for his many valuable suggestions and untiring aid in the direction and completion of the work. He would also thank his former teacher and present esteemed colleague, Professor George H. Meeker, of the Medico-Chirurgical College, for the opportunities which led to its undertaking.

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HISTORICAL REVIEW

The most ancient document containing any reference to the plants of the Myricaceae is the Rhya, a Chinese compilation, written during the Chou dynasty of Tsz Hia, a disciple of Confucius.

To Valerius Cordus (1515-1544), a commentator of Dioscorides, is attributed the first mention in Europe of a plant of this group, made in a simple enumeration of the names of plants in a posthumous publication of Conrad Gesner in 1561 (Gesner "Catalogus Plantarum," p. 31).

Lobel reported in his "Elaeagnos" a plant which he described at length and which is none other than *Myrica Gale*. In the same work Valerius Cordus created the name *Myrica*, but employed it for the designation of *Tamarix*, (p. 68, 1. 12-14).

About the same period Chytraeus, enumerating the plants which grew amongst the heaths of Mecklenburg, cited *Myrica Gale*, which he named *Teutona Myrtus*. The document containing this information is a piece of poetry entitled "Botanoscopium" recently found and published by E. H. L. Krause. (Eine botanische Excursion in d. Rostocker Heide vor 300 Jahr. in Arch. Ver. de Freunde d. Naturg, in Mecklenburg, 1879, p. 329.)

In 1576 Lobel described and figured for the first time the male and female plants of *Myrica Gale* which he named *Gagel Germanorum*. He classed this species in the group of trees and shrubs alongside *Vaccinium* and *Oxycoccos* and stated that it was called by the pharmacists *Myrtus Brabantia*, the Germans naming it Gagel and the English, Goul or Goele. He compared its resinous odor with the perfume of clover, indicated its distribution over heath and forest areas and mentioned that it flourished in England in June and July. (Lobel, Stirpium Observationes, 1576, p. 547.)

In 1586, Dalechamps, in his "Historia generalis Plantarum," p. 110, described and gave illustrations of the same plant which he called *Pseudomyrsine.* He observed it in swampy soil bearing fruits in July and August in the neighborhood of Rouen and indicated that it was known by the name of Royal Pimenta.

In 1616, Rambertus Dodonaeus, in his "Stirpium historia Pemptades," named it *Chamelaeagnus* and classified it under the group of Arbustes non epineux (trees not thorny) between *Rhus Cotinus* and *Viburnum Lantana.* He noticed small drops of resin on the fruit and stated that it was found in Grande-Bretagne, Brabant, Flanders and N. W. France along the Loire.

In 1640, Parkinson ("Theatrum botanicum," p. 1451) grouped it with the Sumacs and named it *Myrtus brabantica aut anglica.* He observed the plant in Sussex, Hertfordshire and Cornwall, England. To the synonyms already mentioned he added those of Sweet Gale and Sweet Willow.

In 1650, Jean Bauhin ("Historia Plantarum," p. 503) wrote that the people of France called it *Gale* after the name given it by Dr. Peter Turnerus.

About the same time, Quatramius observed it in swampy land around Paris and called it *Pigmen*, probably after Pigmentarii.

In 1691, Plukenet in his "Phytographia" illustrated and briefly described one form, the figure of which, in some respect, resembles the fructiferous branch of *Myrica cerifera.* The following is his description "Myrtus Brabanticae accedens Africana, baccis carens Conifera. Arbor Conifera odorata foliis Salicis, rigidis, leviter serratis Ray Hist. an Laur, serrata odora Promont. Bon. Spei Bod a Stapel in Theophr. p. 333, ex America quoque nobis allata est et a Nostratib; Insulam Bermudensem vigentibus, Laurus odorata vulgo nominatur, ut ab honestiss viro Jacobo Harlow didicimus."

A branch of another plant somewhat resembling *Myrica carolinensis* he described as Myrtus Brabanticae similis, Carolinensis, baccifera, fructu racemoso, sessile, monopyrene, fructum fert veluti Saccharo candidiss incrustatum, Cujus ramulus cum fructu ut per Microscopium apparuit adpingitur. Hujus pulchri Sane, perravi et prorsus novi Arbusculi Americani generis, munificentia illustris viri D. Gulielmi Courtene compos factus sum.

In 1693, Plumier ("Description des Plantes de l'Ameriquê") called a species which resembles *Myrica cerifera*, "*Arbor carolinensis* or *Ligustrum americanum lauri folio.*"

In 1695, Petiver in his "Musci Petiveriani" mentions two species, *Gale mariana* asplenii folio now known as *Comptonia asplenifolia*, Ait. and *Gale capensis* now called *Myrica cordifolia* L.

In 1696, Plukenet in his "Almagestum Botanicum" (p. 250) refers to the plant now known by the name, *Comptonia asplenifolia*, (L.) Ait. as *Myrtus Brabanticae affinis* Americana foliorum laciniis Asplenii modo divisis, Julifera simul et fructum ferens."

In 1733, Philip Miller, in Vol. 1, ed. 1 of his Gardener's Dictionary, placed the plants now known as *Myrica Gale* L, *Myrica cerifera* L, and *Myrica Carolinensis* Mill. under the genus *Gale*. He called *M. Gale* of Linnaeus the Sweet Gale, Sweet Willow, or Dutch Myrtle and referred to it as growing upon bogs in several parts of England and casting its leaves in winter. He designated *M. cerifera* L as the Candleberry Tree and *M. carolinensis* Mill. as the Dwarf broad-leaved Candleberry Tree. Both of these plants, he said, were evergreen and indigenous to America.

In his Systema Naturae of 1737, Linnaeus placed the genus *Myrica* in class X, Dioecia and in section IX, Tetrandria, of his sexual system. In applying the term *Myrica* to the plants now recognized as belonging to the *Comptonia* and *Myrica* genera, he revived a name employed a long time before him by Valerius Cordus for the Tamarix.

In 1753, in his "Species Plantarum" ed. 1, p. 1024, he named the tree form with lanceolate shaped leaves *Myrica cerifera*. On different pages of the same volume he gave the names of *Liquidambar peregrina* and *Myrica asplenifolia* to Plukenet's *Myrtus Brabanticae affinis* Americana foliorum laciniis modo divisis.

In his "Spieces Plantarum" 2nd edition of 1763, he classified his earlier *Myrica asplenifolia* under the group Monoecia polyandria and called the plant *Liquidambar asplenifolium*, giving its habitat as North America. In the same volume he classified *Myrica Gale* and *Myrica cerifera* under the group, Dioecia Tetrandria. He described *M. Gale* as having a suffruticose stem with leaves lanceolate in outline and having a sub-serrate margin. He further referred to Peter Kalm's itinerary Vol. 2, p. 312 in which *M. cerifera* is described as a tree with lanceolate, subserrate leaves.

In 1768, Philip Miller named a plant smaller than *M. cerifera* and deciduous in nature, *Myrica Carolinensis*. (Mill. Gard. Dict. ed. 8 n. 3.)

In 1769, Laurent de Jussieu placed these plants in his natural family, Amentiferae, not far from *Betula* and *Castanea*.

In 1771, Catesby ("Natural History of Carolina, Florida and the Bahama Islands," Vol. 1, p. 69.) designated as "Narrow leaved Candleberry Myrtle" what Plukenet in his "Phytographia" and "Almagestum" called *Myrtus Brabanticae similis*, Carolinensis, baccifera, fructu racemoso sessili monopyreno. He described the plant as a small tree or shrub about 12 ft. high with crooked stems branching forth near the ground irregularly, having long, narrow and sharp pointed leaves, some trees having most of their leaves serrated, others not. He also briefly described the condition of the flowers in May, as well as the appearance of the fruits later and stated a method for making candles from the wax. His illustrations of flowering and fruiting branches resemble to a great extent those of *Myrica cerifera* L.

On page 13 of the same work he discussed another plant which he called, "Myrtus Brabanticae similis, Carolinensis, humilior, foliis latioribus et magis serrata, or Broad-leaved Candleberry Myrtle." He further mentions that this plant usually grows not above 3 ft. in height, differing principally from the tall Candleberry Myrtle by its broader leaves. His illustration resembles the fruiting branch of *Myrica Carolinensis* Mill.

In 1778, Lamarck designated as *Myrica palustris*, *Myrica Gale*, of Linnaeus ("Fl. France" 11 p. 236).

In 1789, Aiton, in "Hortus Kewensis" p. 334 divided *M. cerifera* L. into two species, viz.: *M. cerifera angustifolia* and *M. cerifera latifolia*. The latter corresponds to Miller's *Myrica Carolinensis*. He named *Liquidambar asplenifolium* of Linnaeus, *Comptonia asplenifolia*.

In 1794, William Bartram, in the second edition of his "Travels" p. 403, mentioned his excursion to Taensa, Alabama in 1778, where he observed a plant which he named, *Myrica inodora*. He described it as an evergreen shrub, growing in wet sandy soil about the edges of swamps, rising to the height of 9-10 ft., dividing itself into a multitude of nearly erect branches which are garnished with deep green, entire, lanceolate leaves, the branches producing large, round berries covered with a scale or coat of white wax. He further stated that it possessed no fragrance, but was in high esteem among the French inhabitants of this region, who named it the Wax tree on account of its yielding abundant wax for their candles.

In 1802, Loiseleur-Deslongchamps in "Nouv. Duhamel" 2 p. 190, t55 gave the name of *Myrica pensylvanica* to the same species which Miller called *Myrica Carolinensis*.

In 1803, Michaux ("Fl. Am. Bor. 2 p. 228") described a small leaved variety of *M. cerifera* L. which he named var. *pumila*.

In Miller's "Gardener's Dictionary" appearing in 1807, Vol. 2, part 1, nine species of plants are enumerated under the genus *Myrica*. Of those indigenous to the Eastern United States, *Myrica cerifera* is described as rising to a height of 8 to 10 ft. with stiff leaves nearly three inches long and one inch broad in the middle, smooth, entire, of a yellowish-lucid green on the upper side, but paler on the under, alternate, subserrate; male and female flowers on different plants; male catkins about an inch long, erect; female flowers coming out on the side of the branches in long bunches, and succeeded by small rounded berries covered with a sort of meal. *Myrica Carolinensis* is said not to rise as high as *M. cerifera* L. to have branches not so strong and with grayish bark. Its leaves are said to be shorter, broader and serrate, in other respects like those of *M. cerifera* L.

In his "Introduction to Botany" published in 1836, Lindley named the family *Myricaceae*.

In 1864, Casmir De Candolle (Prodromus Vol. 16, p. 147) grouped the genera *Myrica* and *Leitneria* under the family *Myricaceae* and divided the genus *Myrica* into four sections, viz.: Gale, Comptonia, Faya and Subfaya.

Engler in 1894 (Pflanzenfamilien 3 pt. 1, 27) divided the genus *Myrica* into three sections, viz.: Morella, Gale and Comptonia. He stated the characters of each section as follows:

1. Morella:—Flowers dioecious or monoecious, staminate flowers subtended by 2 to 4 or numerous scale like bractlets or ebracteolate; pistillate flowers solitary or in from 2 to 4 flowered clusters, subtended by two bractlets persisting under the fruit. Pericarp, papillose, covered with a warty secretion, or rarely succulent and fleshy, leaves serrate or rarely entire.

2. Gale:—Flowers dioecious; pistillate flowers subtended by two accrescent bractlets, forming lateral wings on the fruit, pericarp smooth and resinous, leaves serrate.

3. Comptonia:—Flowers usually monoecious; pistillate flowers surrounded by 8 linear subulate bractlets, accrescent and forming a spiny involucre in the fruit. Pericarp smooth, resinous and lustrous. Leaves pinnatifid.

Engler joined the family *Myricaceae* to the *Leitneriaceae* to form the alliance of Myricales which he placed after the Piperales and Salicales and before the Balanopsidales and Juglandales.

In 1898, Solereder stated that the family *Myricaceae* included the single genus, *Myrica*. He mentioned the following characters as peculiar to the plants of this genus: Large peltate glands; vertical transcurrence of the smaller veins of the leaf; absence of a characteristic stomatal apparatus; narrow medullary-rays in the wood; tendency to form scalariform perforations in the vessels which never have especially wide lumina; wood prosenchyme with bordered pits; tendency to form a composite and continuous ring of sclerenchyme in the pericycle; superficial formation of cork; oxalate of lime in clustered and solitary crystals; unicellular hairs. (Systematic Anatomy of the Dicotyledons Vol. 2, p. 785.)

The same year, August Chevalier discussed the vegetative organs of the family. ("L'apparail vegetatif des Myricacees," in C. R. Assoc. franc. pour l'avanc. d. Sc., Congres Nantes, p. 457.)

By far the greatest work ever done on the *Myricaceae* was carried out by August Chevalier from 1897 to 1902, whose observations are recorded in a monograph appearing in Mémoires de la Societé Nationale des Sciences Naturelles et Mathematiques de Cherbourg, Vol. 32, p. 85-340, and entitled, "Monographie des Myricacees, Anatomie et Histologie, Organographie, Classification, et Description des Especes, Distribution Geographique." Chevalier's monograph comprises two parts. Part one deals with the general characters of the family. It includes three chapters in which the vegetative apparatus, the root tubercles, and the reproductive apparatus are respectively discussed. Part two embraces the characters of the genera and species, the geographical distribution of the species, and a synthesis of his results.

In this work he divided the *Myricaceae* into three genera, viz:—*Gale*, *Comptonia* and *Myrica*, the differential characters of which I translate from the French in the following table:

Gale	*Comptonia*	*Myrica*
Leaves thin, caducous, entire or feebly dentated.	Leaves thin, caducous, pinnatifid.	Leaves coriaceous, ordinarily persistent, entire, dentated or rarely incised.
Without stipules	With stipules.	Without stipules.
Catkins inserted on the deciduous branches.	Catkins inserted on the deciduous branches.	Catkins inserted on the growing branches.
Dioecious plants.	Dioecious plants.	Dioecious or monoecious plants.
Ordinarily 4 stamens	Ordinarily 4 stamens.	From 4-20 stamens
Very smooth, guarded by 2 entire bracteoles forming aerial buoys.	Ovary smooth, guarded by 2 laciniate bracteoles, provided with emergences at the base and developing itself into a cupule.	Ovary covered with wax bearing or fleshy emergences, either no bracteoles or bracteoles non accrescent.

He remarked that the *Myricaceae* displayed many characters similar to several genera of Piperales and Urticales.

Further reference to Chevalier's monograph, as well as to those of other writers, will be made in the body of this thesis.

COMPARATIVE MORPHOLOGY OF SEEDLINGS

The seedlings studied were in part gathered, and in part grown from seeds planted in the greenhouses of the University of Pennsylvania. On June 20th, 1913, we gathered 28 seedlings of *M. Carolinensis* and 17 of *M. cerifera* at Wildwood, N. J. These varied in age from a few months up to 3 or 4 years of growth. On July 26th, 1914, we collected 15 seedlings of *M. Carolinensis* and 10 of the hybrid *M. cerifera* x *M. Carolinensis*, these ranging from 1 to 4 years of age. On March 21st, 1915, we gathered 15 *seedlings* of M. hybrid at Palermo, N. J. Seeds of *M. Gale*, *M. cerifera*, *M. Carolinensis*, and *M. cerifera* x *M. Carolinensis* (*M. Macfarlanei*) were planted in the University of Pennsylvania greenhouses, but up to the present writing only those of *M. cerifera* have germinated. All of the seedlings collected possessed coralloid clusters of tubercles attached for the greater part to the rootlets coming off from the primary root, but occasionally attached directly to the primary root axis. Examination of 11 seedling root systems of *M. cerifera* grown from seed under artificial conditions revealed the presence of tubercles on all but one.

Gross Structure of Seedlings

Myrica cerifera, L

(Plate 81. Fig. 1)

Primary root, long, flexuose and tapering with numerous slender lateral rootlets, many of which are furnished with tubercles.

Hypocotyl, terete, glabrous, red, somewhat curved in its lower portion and becoming woody very early.

Cotyledons, sessile, glabrous, obovate, opposite, slightly emarginate at apex, clasping at the base, 5.5 mm. long x 4 mm. wide, with a distinct mid-rib, venation pinnate-reticulate.

Epicotyl, erect, terete excepting toward the apex where it becomes triangular, brownish-red to green yellow and red, hairy, glandular, soon becoming woody; first internode 6 mm. long; second 2 to 2.5 mm.; third 6 mm.

Leaves, simple, cauline or ramal, first and second pairs opposite, the rest alternate, petiolate, alternately pinnately nerved, the lower ones thin hairy on upper surface and margin, the upper devoid of simple hairs with the exception of the margin and petiole and mid-rib portions which possess even fewer thin simple hairs than *Myrica Carolinensis*, yellow and reddish, glandular on both surfaces, petiole flat on upper surface and somewhat dilated at its junction with the stem, arrangement 2/5 spiral.

First pair of foliage leaves: Opposite, obovate, cuneate, tricleft, apex mucronate, base oblique, margin entire in lower 2/3, serrate in upper 1/3, venation alternately pinnate-reticulate, secondary nerves coming off at angle of 45°, short petiolate.

Second pair of foliage leaves: Opposite, obovate-cuneate, tricleft, apex mucronate, with terminal lobe entire or showing 1-2 serrations, lateral lobes beginning to show 1 or 2 teeth, petiole very slightly longer than that of first pair.

Third leaf: Similar to second leaf, but petiole slightly longer and lateral lobes distinctly 2 toothed.

As we pass upward along the seedling axis we find the leaf margin becoming more and more serrated until the highest leaves exhibit serrations in their upper half, rarely in their upper 2/3. Occasionally we have found one inequilateral leaf among the upper group.

Myrica Carolinensis, Miller
(Plate 81. Fig. 2.)

Primary root and hypoctyl similar to those of *Myrica cerifera* seedling. Cotyledons, sessile, glabrous, opposite, obtuse and slightly emarginate at the apex, somewhat clasping at the base, 5 mm. long x 3 mm. wide; venation pinnate-reticulate with a distinct mid-rib.

Epicotyl, erect, terete, excepting toward apex where it gradually becomes triangular, brownish-green to green, hairy, yellow glandular, soon becoming woody; first internode 6 1/2 mm. long, second 3 1/2 mm.; third 2 mm.

Leaves, simple, cauline or ramal, first pair opposite, the rest alternate, petiolate, alternately pinnately nerved, slightly thinner than those of *M. cerifera*, thin hairy on both upper and lower surface and margin, yellow glandular on both surfaces, the glands on the lower surface being more numerous than on the upper, petiole flat on upper surface, slightly dilated at its insertion on the stem. Arrangement 2/5 spiral.

First pair of foliage leaves: Opposite, obovate, tricleft, apex mucronate, base oblique, margin entire in lower 2/3, serrate in upper 1/3, venation alternately pinnate-reticulate, secondary veins coming off at angle of 45°, short petiolate.

Second foliage leaf: Alternate with first pair and third leaf, pinnately tricleft with terminal lobe entire or showing one serration, lateral lobes beginning to show two teeth.

Third foliage leaf: Alternate with second and fourth, petiole slightly longer than second, lateral lobes distinctly two-toothed.

As we pass upward along the seedling axis we find the leaf margin gradually becoming more and more serrate until the highest leaves are generally serrate in their upper 1/2. We also find that the number of glandular hairs on the upper surface gradually diminishes until some of the highermost inserted leaves are entirely devoid of them on this area.

Myrica Macfarlanei, (Youngken): (*M. cerifera* x *M. Carolinensis*)
(Plate 81. Fig. 3)

Primary root and hypocotyl similar to those of *M. cerifera* and *M. Carolinensis.* Epicotyl, erect, terete excepting toward apex, where it becomes triangular, brownish-green to green, hairy, yellow and red glandular soon becoming woody and branched; first internode 5 mm. long, second 3 1/2 mm.; third 1.75 mm.

Leaves simple, cauline or ramal, 1st pair opposite, the rest alternate, venation pinnate-reticulate, secondary veins coming off from mid-rib at angle of 45°, about the same texture as those of *M. cerifera*, petiolate, thin hairy on margin and upper surface over veins, yellow to red glandular, but glandular hairs more frequent on lower than upper surface, and yellow glands alone present on upper surface, petiole flat on upper surface and showing numerous hairs, somewhat dilated at its insertion on the stem. Arrangement 2/5 spiral. While the leaves of the seedling hybrid remain green during the summer and early autumn, they gradually become discolored during the late autumn and winter, most of them remaining on the stem and branches until the new leaves bud forth in the spring.

Of all the hybrid seedlings examined at Palermo, March 21st, 1915, we found most of the leaves present and having a reddish brown color; a few were only partly discolored.

First pair of foliage leaves: Opposite, obovate-cuneate to rarely orbicular in outline, tricleft, apex mucronate, base more or less oblique to seldom rounded, margin entire in lower ⅔, serrate in upper ⅓, venation alternately pinnate-reticulate, extremely short petiolate.

Second foliage leaf: Alternate with first pair and third leaf, obovate-cuneate, apex either 3-5 toothed or 3 lobed, each lobe being mucronate at its apex, base oblique, margin below apical region entire and hairy, venation similar to first pair of leaves, somewhat longer petioled than first pair. Upper surface with simple hairs often losing these in winter, few or no yellow glandular hairs. Lower surface hairy along mid-rib few or no simple hairs elsewhere. Both yellow and red colored glandular hairs present on this surface.

Third foliage leaf: Alternate with second and fourth leaf, obovate-cuneate, tricleft, apex mucronate, lateral lobes bi-dentate or one tooth appearing below each lobe, larger petioled than second leaf, in other respects resembling it. The leaves become more and more serrate in ascending the axis until the higher ones are reached which are serrate in their upper halves.

Histology of Seedlings

Root

The roots of primary growth of *M. cerifera*, *M. Carolinensis* and *M. Macfarlanei* are very similar in their minute structure. In transverse section passing from periphery toward the centre, one observes the following structures:

1. An epidermis of brick shaped cells whose outer walls are cutinized and whose contents are protoplasmic. Numerous thin walled root hairs arise as outgrowths of this tissue for a considerable distance above the short root cap.

2. Cortex of about 5 layers of radially arranged rounded cells showing large intercellular-air-spaces with the exception of the layer just beneath the epidermis. Calcium oxalate crystals of the rosette type are found in a few of the cells.

3. Endodermis of a single layer of typical endodermal cells, the walls of which become thickened very early and whose contents then consist largely of a brownish substance which Chevalier calls "lignine-gommeuse."

4. Pericambium of 1-2 layers of large thin walled cells rich in protoplasm.

5. Radial fibro-vascular bundle which is tetrarch.

6. Pith in centre very small and composed of mostly polygonal cells with punctations on their radial walls. Between the various pith cells are very small intercellular-air-spaces.

Epicotyl

In all three of these seedlings a transverse section made midway between the cotyledons and first pair of foliage leaves shows the following structural detail:

1. Epidermis of polygonal cells highly cutinized on their outer face and giving rise to short and long, frequently bent, unicellular trichomes and short stalked glandular trichomes with balloon-shaped heads (*M. Carolinensis* and *M. Macfarlanei*) and both bowl- and balloon-shaped heads (*M. cerifera*).

2. Cortex of 6-8 layers of radially arranged polygonal cells, larger in the meso- and endo- than in the exocortex region. Many of these cells contain tannin, some starch grains, others rosette and monoclinic crystals, a few gummy lignin.

3. Endodermis not distinguishable from the other cells of the endocortex.

4. Pericambium showing a discontinuous ring of sclerenchyma elements.

5. Open collateral fibro-vascular bundles whose phloem region is very narrow and composed mostly of sieve tubes and phloem cells. The phloem cells are loaded with tannin.

6. A rectangular pith zone of polyhedral parenchyma cells whose walls are thickened. Many of these cells contain starch and a few gummy lignin.

First pair of foliage leaves of *M. cerifera*

The upper epidermis is composed of cells with slightly wavy vertical walls having a thicker cuticle than those of the lower epidermis and showing no stomata. In surface view these cells have a mean measurement of 33.99 μ × 26.22 μ.

The cells of the lower epidermis have more prominently wavy vertical walls with a mean measurement of 36.42 μ × 24.28 μ. The cells over the veins possess rectilinear walls and are somewhat elongated. Among the lower epidermal cells are to be noted many stomata which are slightly elevated and surrounded by 3-8 neighboring cells.

The mesophyll shows dorso-ventral differentiation into palisade and spongy parenchyma regions. The palisade zone consists of two layers. The upper one of distinctly columnar shaped cells, the lower of both columnar and cuboidal cells which for the most part are more loosely arranged and smaller than those of the upper layer. The spongy parenchyma region consists of small rounded to irregular shaped cells which surround large intercellular air spaces. A few of these cells are filled with gummy lignin, probably due to the invasion of the endophyte which will be discussed later.

The mid rib and lateral veins are found coursing through this region. The mid rib shows 1 or 2 layers of schlerenchyma elements bordering its lower face. Many of the mesophyll cells bordering the mid rib and lateral veins show rosettes of calcium oxalate. Both surfaces show numerous crypts containing glandular peltate hairs of two kinds, viz.: golden yellow and reddish. Of these, the golden yellow variety are by far more numerous. They consist of a pedicel or stalk two layers thick and 3-4 cells high with a balloon-shaped multi-cellular head containing oil and resin. The reddish variety are by far fewer and possess a stalk portion four layers thick and two cells high with a bowl-shaped multicellular head containing a small mass of resin. Simple unicellular trichomes are found on both lower and upper epidermis.

First pair of foliage leaves of *M. Carolinensis*

The upper epidermis is composed of cells whose vertical walls appear more wavy in surface view than those of *M. cerifera*. The outer walls of these cells have a mean measurement of 63.34 μ x 33.46 μ. The cells directly over the veins show rectilinear walls and are slightly elongated in the direction of the vein.

The lower epidermis possesses cells whose vertical walls are indeed more wavy than those of the upper epidermis and whose outer walls show a mean measurement of 54.87 μ x 23.30 μ. Numerous stomata are present among the epidermal cells of this surface. These are surrounded by 5-8 neighboring cells.

The mesophyll resembles that seen in the first pair of foliage leaves of *M. cerifera* with the exception that it is only about half as wide, shows more loosely arranged palisade cells, and has fewer scelerenchyma elements below the mid rib and nerves.

The upper surface shows fewer crypts than that of *M. cerifera* and both surfaces are devoid of reddish glandular hairs with bowl-shaped heads. Both surfaces however possess the golden-yellow glandular hairs with balloon-shaped heads. The unicellular simple hairs are more numerous than on either *M. cerifera* or the hybrid (*M. cerifera* x *M. Carolinensis*).

First pair of foliage leaves of *M. Macfarlanei* (*M. cerifera* x *M. Carolinensis*

The first pair of foliage leaves of the hybrid shows histological peculiarities which in several respects are intermediate with those of both of its parents—*M. cerifera* and *M. Carolinensis*. For instance, the cells of the upper epidermis in surface view have their vertical walls more wavy than those of *M. cerifera*, less wavy than those of *M. Carolinensis*, Their outer walls have a mean measurement of 41.27 μ x 26.95 μ, while those of *M. cerifera* are 33.99 μ x 26.22 μ and of *M. Carolinensis*, 63.34 μ x 33.46 μ; the mesophyll region is narrower than that of *M. cerifera*, broader than that of *M. Carolinensis*; the lower epidermis has cells whose outer walls in surface view show a mean measurement of 37.87 μ x 21.85 μ, while those of *M. cerifera* are 36.42 x 24.28 and *M. Carolinensis* 54.87 μ x 23.30 μ; fewer unicellular simple trichomes on upper epidermis than those of *M. Carolinensis*, more than those of *M. cerifera*.

They resemble those of *M. cerifera* in that both red and golden-yellow headed glandular hairs are present on the lower surface and also by the fact that their upper epidermis has a cuticle of about equal thickness.

They resemble *M. Carolinensis* in that they have few crypts on the upper epidermis containing golden yellow glandular hairs. The mid rib and stronger veins are completely surrounded by a zone of sclerenchyme and connected with the upper epidermis by collenchymatic cells.

COMPARATIVE MORPHOLOGY OF THE ROOTS

The roots of *M. cerifera*, *M. Carolinensis*, *M. Macfarlanei*, *M. Gale* and *Comptonia asplenifolia* show the general structure of Dicotyledons. All of the younger ones which the writer has examined possessed a root cap, above which were noted numerous root hairs with the exception of those of M. Gale which possessed relatively few.

The subapical region showed three groups of generative tissues which form the calyptra and epidermis, cortex, and central cylinder.

The radicle grows downward in a rather tortuous manner and forms the fibrous tap root, which in the above named species is soon exceeded by the lateral roots borne on the hypocotyl axis.

Primary Structure

The piliferous axis is composed of large clear pavement like cells, which frequently elongate into root hairs. The cortex is composed of 5-12 layers of cells, the most external layer of which is deprived of intercellular air spaces and collenchymatic. The other layers are composed of rounded to polygonal cells arranged in somewhat loose radial rows extending to the endodermis. The air spaces in *M. Gale* and *M. Carolinensis* growing in humid earth are very large. Some of the cells of this region contain rosettes of calcium oxalate, others tannin, occasionally gummy lignin.

The endodermis consists of typical endodermal cells. It dies very early and becomes filled up with a brownish substance which Chevalier calls "ligneux-gommeuse" (gummy lignin).

The pericambium is composed of one or two layers of large meristematic cells which frequently give rise to side rootlets. The radial bundle, according to Chevalier, (7, p. 97) possesses 4-8 tracheal poles, the number varying in the same species. The writer has found the bundle to be tetrarch in *M. cerifera, M. Carolinensis, M. Macfarlanei* and *Comtonia asplenifolia.*

The pith region is composed of polyhedral cells with simple punctations on their transverse walls, and possessing small air spaces. Transition:—The external layer of the pericambium very early becomes a cork cambium laying down cork on its outer face, thus cutting off the nutrient supply and causing the tissues beyond e.g., epidermis, primary cortex and endodermis to sluff off. On its inner face the cork cambium cuts off secondary cortex. Secondary meristem develops on the inner face of the phloem patches forming intrafascicular cambium as well as on the outer face of the xylem strands forming there interfascicular cambium and so a continuous cambium zone gradually arises which cuts off secondary phloem on its outer face, thereby adding meta-phloem and cuts off secondary xylem on its inner face thus augmenting the total mass of xylem tissue. Secondary medullary rays are also cut off by the cambium. The original radial structure of the stele changes to the open collateral type.

The primary phloem soon becomes inactive; the cells have their walls thickened and frequently appear collenchymatic.

Secondary Structure

Myrica cerifera, Linnaeus. (Plate 86. Fig. 16.)

In passing from periphery toward the centre the following peculiarities are to be observed:—

1. Cork of several layers of irregular brick-shaped cells which vary in their staining capacity. Many of the cells have highly suberized walls which stain green with extract of chlorophyll. Some, however, have all of their walls lignified, while others show lignification only on their inner walls. The lignification of these was readily determined through the use of phloroglucin and hydrochloric acid which imparted the characteristic pink coloration.

2. Phellogen of meristematic cells in a rapid state of division.

3. Secondary cortex whose cells are tangentially elongated and smallest in the outermost portion, becoming larger as one passes toward the phloem. Usually three or four of the outer layers of the exocortex region are devoid of intercellular air spaces. The remaining layers show numerous air spaces which are for the greater part small and angular. Most of the cortical parenchyme cells contain spheroidal starch grains with a central cleft hilum, either simple or 3-compound. Some, however, contain rosette aggregates and monoclinic prisms of calcium oxalate while others have their walls thickened and are filled with a yellowish-brown substance which presents the following reactions: It is insoluble in cold or boiling water, cold concentrated potash solution, ammonia, alcohol or xylol. It is soluble in boiling nitric acid, boiling concentrated potash solution and hot solution of sodium hypochlorite. This substance has been termed "lignine-gommeuse" by Tison (8) and Chevalier, (7 p. 133).

Sclerenchyme fibres which are quite narrow are few and usually isolated singly or in small groups of 2, 3 or rarely 4 amongst the cells of the cortex.

4. Endodermis whose cells do not differ materially from those of the adjacent cortex.

5. Fibro-vascular bundles of the open-collateral type, consisting of phloem, cambium and xylem regions separated by medullary-rays. The phloem region is comparatively wide. Many of the medullary-rays in this region are broadened out in fan-shaped fashion. The bast fibres are arranged singly or in groups forming interrupted circles. They are more numerous in the metaphloem than in the protophloem region. Crystal-fibres containing monoclinic prisms of calcium oxalate accompany the bast fibres. Starch grains and tannin are found both in the phloem cells and the phloem medullary rays. Many of the phloem cells and air spaces contain monoclinic prisms of calcium oxalate. Frequently several crystals are present in one cell or space.

The xylem is porous and traversed by numerous medullary-rays, continuous with those of the phloem. The medullary-ray cells of this region are more radially elongated and thickened than those of the phloem, but resemble the latter in their content.

The tracheae are more or less polygonal in transverse sections and show barred septa. Some of them contain *Actinomyces* which has found its way into them from the tubercles. This organism is always associated with debris, presumably due to its eroding action upon the tracheal walls. Tyloses are frequent. The walls of the tracheae are pitted. Solereder errs in stating that the maximum diameter of the vessels varies between .02 and .05 mm. (9) In this root, the writer has found it to be .096 mm. The woody fibres are extremely elongated and taper-ended. Their walls are considerably thickened with deposits of lignin and show oblique pores. The wood parenchyma cells are relatively few and contain starch and frequently monoclinic prisms of calcium oxalate. The primary medullary rays are mostly 1-4 cells, rarely 1-5 cells, broad. The secondary medullary rays are 1-2 cells broad.

Myrica Carolinensis, Miller. (Plate 86. Fig. 14.)

The root of *M. Carolinensis* resembles that of *M. cerifera* in respect to tissue arrangement, character of cork, cells containing "lignine-gommeuse" and tracheae, some of which contain *Actinomyces.* It differs from *M. cerifera* root in the following particulars: The cortex shows larger intercellular air-spaces with more crystals of calcium oxalate.

The sclerenchyme elements are more numerous and usually in groups of several, forming small islets.

The phloem contains fewer bast fibres and for the most part broader medullary rays. Many of the latter are 1-5 or 1-6 rows of cells in width.

The tracheae are less numerous while the woody fibres are more abundant. The tracheae are to some extent narrower and show both pitted and reticulate markings on their walls.

The primary medullary-rays are wider and more numerous. They range from 1-5 to 1-6 rows of cells broad. The cells of both the primary and secondary medullary rays are somewhat broader and shorter.

Myrica Macfarlanei, Youngken. (*M. cerifera* x *M. Carolinensis*)

The root of this hybrid between *M. cerifera* and *M. Carolinensis* shows characters some of which resemble both parents, some, one parent and others which are peculiar to itself.

It resembles both parents in respect to its cork, its cells containing gummy lignin, *Actinomyces* in ducts, barred septa, and crystal fibres accompanying the hard bast.

It resembles *M. Carolinensis* in the number of primary medullary-rays and in having comparatively broad medullary-ray cells.

It differs from both parents by having larger intercellular air spaces in the cortex, more crystals of calcium oxalate in both the cortex and phloem, broader and more numerous sclerenchyme elements in the cortex, and narrower tracheae. (Plate 86. Fig. 15.)

The primary medullary-rays are 1-6, occasionally 1-7 rows of cells in width.

Myrica Gale, L. (Plate 86, Fig. 17.)

In passing from periphery toward the centre, the following structures are to be observed.

1. Cork of several layers of elongate brick-shaped cells which are unevenly stained and whose walls are partially lignified, in part suberized.

2. Cork cambium of tangentially elongate meristematic cells.

3. Cortex of a few layers of tangentially elongated parenchyma cells with small angular intercellular air-spaces. Most of the cells of this region contain starch and tannin, many possess conglomerate and monoclinic prisms of calcium oxalate, while a few have their cell walls thickened and contain gummy lignin. No sclerenchyme elements are present.

4. Endodermis whose cells do not differ materially from those of the adjacent cortex.

5. Phloem which is comparatively narrow and composed solely of sieve tubes, companion cells and phloem parenchyme. The primary medullary-rays in this region are broadened out in fan-shaped manner and show a width of 1-5 rows of cells.

6. Cambium of thin walled tangentially elongated cells.

7. Xylem which is very compact in nature showing fewer tracheae than in any other *Myrica* root found in the Northeastern states. Most of the tracheae are distinctly polygonal and form interrupted rings in the spring wood. (Plate 86. Fig. 17.) Their walls show transverse pits which are usually arranged in longitudinal rows. The greater part of the xylem consists of tracheids which are square in transverse section. The primary medullary-rays are 1-4 rows of cells wide and are always four in number. They are arranged in a cruciform manner. The secondary medullary-rays are 1-2 rows of cells wide. Both kinds of medullary-rays contain numerous spheroidal starch grains. Tannin is found in considerable quantity in the cortex, phloem and medullary rays.

Comptonia asplenifolia, (L.) Aiton. (Plate 86. Fig. 18.)

This root presents the following characteristics:—

A cork of several layers of reddish-brown, brick-shaped cells whose walls are for the greater part suberized. Beneath the cork is found a phellogen zone of somewhat tangentially elongated cells which cut off cork in an uneven manner.

The cortex is comparatively narrow and is composed of tangentially elongated parenchyma cells, most of which contain starch, some rosettes and monoclinic prisms of calcium oxalate, while a very few contain gummy lignin. Sclerenchyme fibres are rare in this region.

The phloem in relation to the cortex is comparatively broad. It contains, besides soft bast, numerous islets of bast figures which are accompanied by crystal fibres. The crystal fibres are composed of rows of superimposed thin walled parenchyme cells, some of which contain rosettes, others, monoclinic prisms of calcium oxalate. The primary medullary-rays in this region are numerous and are 1-5 rows of cells wide. They broaden out toward the cortex in fan-shaped fashion.

The cambium zone is distinctly wavy in character.

The xylem is traversed by numerous primary and secondary medullary rays which are continuous with those of the phloem region. The cells of these contain numerous starch grains and considerable tannin. The tracheae are very numerous, occupying most of the wood area. Their walls are pitted. No barred septa have been observed. The woody fibres are generally quite narrow, taper-ended and extremely lignified.

The secondary medullary-rays are from 1-2 to 1-3 cells wide, while the primary rays vary from 1-4 to 1-5 cells in width.

Tannin is present in cortex, phloem and medullary-rays.

COMPARATIVE MORPHOLOGY OF STEMS

Gross Structure

Myrica cerifera, L.

A shrub or tree from 1 to 12 m. high, erect to ascending in habit and having a tall crooked trunk 20 to 30 cm. in diameter, and upright or spreading crooked branches which form a round-topped head. The recent shoots are greenish to reddish brown, twigy-elongate at the ends of branches or branchlets and bearing numerous leaf buds above and flower buds below during the season The upper portions of these shoots are densely covered with yellowish- and orange-red glands and a sparse number of whitish non-glandular hairs. The lower portion has fewer glands and hairs. Many reddish-white to white, raised, oval, spotlike lenticels are scattered over the surface. These are slightly elongated longitudinally and show a length of 5 mm. The branchlets of second year's growth are reddish-brown and show a few glands but no non-glandular hairs. The lenticels are larger, arranged longitudinally and vary from .5 to 1.5 mm. in length. The branchlets in their lower

2/3 bear either male or female catkins. The older branches are grayish in color. The bark of the trunk is about 6 mm. thick and has a smooth close light gray surface. The plant is frequently a shrub from .5 to 3 m. high sending up numerous crooked branches from near the ground. The underground branches grow horizontally for long distances through the soil and give off numerous tufts of suckers which arch upward.

Myrica cerifera var. pumila, Michaux

A low much branched shrub 2-6 dm. tall, having erect or ascending stems which are frequently tufted.[10] The branchlets of first year's growth are occasionally extremely hairy, frequently polished and shining. Other features of the branches are similar to those of *M. cerifera.*

Myrica Carolinensis, Miller

A shrub attaining a height of 2 to 3 m. with crooked stems which branch frequently. The recent shoots are greenish to dull reddish-brown and are covered with yellow glands and shaggy white non-glandular hairs, the former being more numerous toward the summit. The non-glandular hairs are far more numerous than on similar shoots of *M. cerifera.* Both leaf and flower buds are borne on these shoots, the former always above the latter. The twigs are stiff at the ends of the shoots of last year's growth.

The branchlets of second year's growth are purplish-brown and show numerous small whitish to grayish lenticels which vary from .5 to 1 mm. in length. These branchlets bear either staminate or pistillate catkins in their lower 2/3. The fruit bearing axes persist on the stem of the third year's growth. On January 31, 1915, the writer found them also present on the stems of the 4th, 5th and 6th year of one plant growing in the open. The older branches are ash-brown to ash-gray in color, reticulately wrinkled and show numerous transversely elongated lenticels The underground branches form crooked scaly rhizomes which creep through the soil in a horizontal manner and give off numerous lateral branches. They send up tufts of spreading suckers at different nodes which frequently serve to propagate the species. These suckers grow downward and then upward in arcuate fashion. They are of a whitish to reddish-white aspect when recently dug up.

Myrica Macfarlanei, Youngken. (*M. cerifera* x *M. Carolinensis*)

A shrub rising to a height of 2 to 2.5 m. with very crooked branches, the younger of which frequently appear stunted in habit. The shoots of the first year's growth are somewhat intermediate in nature with those of *M. cerifera* and *M. Carolinensis.* They are of a greenish to reddish-brown color, more thin hairy than those of *M. cerifera*, somewhat less hairy than those of *M. Carolinensis*, but showing numerous yellow and a few orange-red glands. Upon them appear the buds of next season's leaves and flowers. The branchlets of two years' growth are dull reddish-brown, and devoid of thin hairs but possess a few golden-yellow glands. Lenticels are present which vary from .5 to .7 mm. in length and are arranged longitudinally. Alike with similar branchlets of *M. cerifera* and *M. Carolinensis*, staminate or pistillate flowers are borne on special catkin axes which, in the case of the pistillate, persist for a long time after the fruits have fallen. The writer observed these also present on stems of the 3d, 4th and 5th year's growth at Wildwood, January 31, 1915 (Plate 85, Fig. 13). The older stems are brownish gray to ash gray in color and have numerous circular to oval somewhat raised lenticels, arranged both longitudinally and transversely and varying from .5 to 1.5 mm. in length. The underground stem creeps through the soil for long distances and gives off numerous branches which are similar in their habit to those of *M. cerifera* and *M. Carolinensis.*

Myrica Gale, L.

A dark looking shrub rising to the height of from .4 to 2 m. and dividing into several slender branches which in turn divide and redivide in furchate manner. The recent shoots are of a rich dark purple color, polished and shining and bear numerous thin white hairs and yellow glands which are most numerous toward their summit. The lenticels on these shoots are relatively few, of a white color, and circular in shape having a diameter of .25 mm. The branchlets of second year's growth are also dark purple, showing polished and shining circular to oval, whitish lenticels, which vary in diameter or length from .35 to .5 mm. The older branchlets and branches are reddish brown in color and usually polished. They show elevated, transversely-elongated lenticels which vary from .5 to 2 mm. in length. On the main and lowest aerial stem the outer bark cracks and rolls horizontally becoming rough, but still is somewhat shiny. Special branches are developed on the young shoots which bear the catkins. These die after the descent of the pollen. The rhizome creeps through the soil for long distances and bears numerous lateral branches and suckers. The latter are reddish to reddish-purple in color and bear thick pink to pinkish-white scales.

Myrica inodora, Bartram

A shrub or small tree attaining a height of 6 mm., with a maximum trunk diameter of nearly 9 cm(10). The first branches are ascending, often straight with a white bark. The branchlets of recent growth are smooth and reddish brown with a few longitudinally elongated lenticels, 5 mm. long. Those of the second year's growth are ash brown in color and show lenticels 5 to 7 mm. long.

Comptonia asplenifolia, L. Aiton

A round-headed low shrub growing to the height of 1 to 1.25 m. The main aerial stem is ascending in habit and grows to the length of about 3 dm. It frequently bifurcates into two crooked branches which bear numerous branchlets toward their summit. The recent shoots are greenish yellow to reddish brown in color and covered with long white hairs and minute yellow shining glands which are always more numerous toward the ends of the branches. Usually one or two thin special branches are borne at the ends of these which bear catkins. These die after the descent of the pollen but often remain on the shoots until the next season, especially in localities where the plant is well protected from winds. The branchlets of the 2d and 3d year's growth are of a steel to reddish-brown color, somewhat polished and show scattered transversely-elongated lenticels which vary from .5 to 1 mm. in length. The older branches become darker in color but retain their reddish aspect. These are usually quite crooked and show narrower transversely elongated lenticels from 1-3 mm. in length. The main stem shows a dark and considerably cracked cork. The underground stem is extremely crooked and creeps through the soil for a long distance giving rise to numerous branches and suckers at its various nodes. The suckers branch downward then upward forming inverted arches (Plate 82, Fig. 4). It possesses relatively few lenticels which are not raised like those of the aerial stems. Some of the underground stems bear tubercles, others thin fibrous roots upon which the tubercles are clustered.

Histology

Under this sub-caption the microscopic peculiarities of stems of primary growth, showing primary structure, will first be considered from a general standpoint. Those of aerial and subterranean branches of secondary growth showing secondary structure will be specifically treated in the following order: (a) *M. cerifera*, (b) *M. Carolinensis*, (c) *M. Macfarlanei*, (d) *M. Gale*, (e) *Comptonia asplenifolia*.

Primary Structure
(*M. cerifera*, *M. Carolinensis*, *M. Macfarlanei*, *M. Gale*, and *Comptonia asplenifolia*)

The epidermis of the young stems is covered by a cuticle more or less thickened according to the species. The stomata are comparatively few and frequently elevated. Hairs analagous to those of the leaves are found in large numbers and are especially numerous on the distal extremities (*vide supra*).

The cortical parenchyme is composed externally of cells containing chloroplasts. Internal to these are cells which during periods of rest contain many starch grains. Certain thin walled cells are very rich in tannin content, which may be readily determined by the use of ferric chloride solution which imparts a bluish-black color to this substance. Other cells of this region contain crystals of calcium oxalate in the form of rosettes, tabular monoclinic prisms, or crystal sand. Hambright and Moore have found in the cortex of *Myrica* species traces of gum, resin, volatile oil, palmitic and myristic acids. Beringer has observed and figured "secretory resin cells" in the cortex of *Comptonia asplenifolia*. Chevalier (7, p. 103) and the writer have found these "secretory resin cells" of Beringer to be nothing other than dead cells filled with gummy lignin.

The pericambium consists of polyhedral cells of uniform size and formed opposite each of the fibro-vascular patches. Very early many cells of this region become thickened in their walls through the deposition of lignin. The primary conducting system is composed of 10 cauline fibro-vascular bundles which form in the internode a lobed gamostelic ring. These bundles break up in the vicinity of the nodes to furnish 3 veins running into each leaf. In the crown one is able to distinguish up to 19 apparent conducting masses separated by very large medullary-rays. At each node, the crown bundles split up in the region of leaf insertion. At first a large cord of wood and bast becomes detached, then, a little higher two smaller cords separate, which take up their position, respectively, to the right and to the left of the first. The gamostele closes up after first filling in the gap left by the departure of the lateral bundles.

In each procambial cord the first phloem elements consisting of sieve tubes, companion cells and phloem parenchyme cells may be distinguished in contact with the pericambial arc. The primitive tracheae appear later in contact with the pith parenchyme. The woody fibre differentiation of the procambial parenchyme, corresponding to the bast fibre elements takes place subsequently (7 p. 100). The external layer of the cortex gives rise to the cork cambium.

Secondary Structure
Aerial Stem of *M. cerifera*, L.
(Plate 87, Fig. 19)

The cork consists of several layers of brick-shaped cells, whose walls are for the greater part suberized. The contents of these cells stain variously with safranin and methyl-green.

Separating the cork from the cortex, one notes a phellogen layer of tangentially elongated cells. The cortex is usually narrow and shows comparatively small angular intercellular air spaces. Its cells are tangentially elongated. Some of them contain gummy lignin and tannin, while many are filled with starch grains, or crystals of calcium oxalate which may be in the form of monoclinic prisms or rosette aggregates. Occasionally these crystals may also be seen in the air-spaces. Stone cells of irregular form and size, arranged singly or in small groups, are scattered throughout this region. The outer 3 or 4 layers of exocortex are composed of collenchymatic cells and show no air-spaces. A continuous zone of sclerenchyme fibres and cells is present in the pericycle. The sclerenchyme cells are of different sizes and shapes and show varying degrees of lignification. Frequently cells containing crystals of calcium oxalate are found adhering to them. Some of the stone cells contain gummy lignin. The phloem region is traversed by numerous primary and secondary medullary-rays which generally broaden out toward the the cortex, arching in many phloem masses. The primary medullary-rays are usually 1-3 rows of cells in width in this region. The secondary medullary-rays of the phloem vary from 1-2 rows of cells in breadth. The sieve tubes show oblique sieve plates. Their walls show numerous transverse pits. Tannin and crystals are quite abundant in the phloem parenchyma. Both stone cells and narrow bast fibres are sparsely scattered either singly or in small groups amongst the other phloem elements. The bast fibres are accompanied by crystal fibres. Each cell of the crystal fibres contains a monoclinic prism of calcium oxalate. The cambium zone is distinct and somewhat wavy. The xylem region is traversed by numerous primary and secondary medullary-rays which separate this zone into many narrow wood wedges. The primary medullary-rays of this region are mostly 1-2, rarely 1-3 rows of cells wide, the secondary, mostly 1 cell, rarely 2 rows wide. The walls of the medullary-ray cells are lignified. The tracheae are pitted and appear circular to very slightly angular in transverse section. Some of them show the presence of the *Actinomyces* organism. Alike with those of the root, they exhibit barred septa. Their distribution in the spring, summer and autumn wood is strikingly uniform. The numerous wood fibres are narrow, elongate and taper ended. Their walls show many oblique pits. The wood parenchyma cells are elongated in the direction of the long axis of the stem and show slightly thickened walls.

The pith region is composed of rounded to polygonal parenchyma cells, whose walls become lignified very early. Some of them contain a brownish-yellow substance (gummy lignin) while others contain starch grains. Protoplasmic processes extending from cell to cell are quite prominent.

Subterranean Branch of *Myrica cerifera*, L.

The rhizome of *Myrica cerifera* differs histologically from the aerial stem in the following particulars:—There are fewer layers of cork and more layers of cortical parenchyme cells. In transverse section the cortex cells appear more rounded, less tangentially elongated. The intercellular air-spaces are somewhat larger. No crystals of calcium oxalate have been found either in the cortex or pith. There are generally more cells containing gummy lignin. Sclerenchyme elements are entirely wanting in the cortex. A discontinuous zone of sclerenchyme is present in the pericycle, composed of numerous small islets of pericambial fibres. The phloem region shows fewer bast fibres. The medullary-ray cells are broader and have thinner walls. The tracheae of the xylem are fewer and for the most part about twice as broad. The largest have a maximum breadth of 57.6 μ, while the largest of the aerial stem are 38.4 μ. In some of these the writer has observed colonies of coccus-like structures which probably represent an involution form of the *Actinomyces* previously discussed. The pith cells are about twice as large, contain more starch and have considerably thinner walls.

Aerial stem of *Myrica Carolinensis*, Miller

Passing from periphery toward the centre the following structures can be observed:—

1. Cork of several layers of brick-shaped cells, whose cell walls are either suberized or lignified or in part suberized and in part lignified. There is a tendency for more lignification to occur in this region than in that of the aerial stem of *M. cerifera*.

2. Phellogen of a single layer of thin-walled tangentially elongated cells.

3. Cortex generally broader than corresponding region of *M. cerifera* of same age. Its cells are tangentially elongate and contain either starch grains, gummy lignin or crystals of calcium oxalate either in the form of rosette crystals or monoclinic prisms. The intercellular air-spaces are generally somewhat larger than those of the cortex of *M. cerifera* aerial stem and frequently contain several crystals lying in a row one above the other. Stone cells are quite numerous and scattered either singly or in groups throughout the cortical parenchyme.

4. The pericycle shows a broken ring of sclerenchyme elements. These are for the most part considerably narrower than those in the stem of *M. cerifera.*

5. The phloem is uniformly narrower than the similar region of *M. cerifera.* Through it run numerous medullary-rays. The phloem elements are smaller than those of *M. cerifera.* Many of the phloem cells contain rosettes or monoclinic prisms of calcium oxalate. Tannin is also present in large amounts Few bast fibres occur in this region.

6. The cambium line is much less wavy than that of *M. cerifera.*

7. The xylem consists of numerous tracheae containing *Actinomyces* forms intermingled with many woody fibres and comparatively fewer wood parenchyma elements The tracheae are generally narrower than those of *M. cerifera* aerial stem. The largest have a measurement of 38.4 μ across, similar to the largest in the aerial stem of *M. cerifera.* The autumn wood is almost wholly composed of greatly thickened woody fibres which collectively form a band in each annual ring fully twice as wide as that observed in *M. cerifera.* (Plate 87, Fig. 21.)

The medullary-rays are thinner than those of *M. cerifera.* This is not due to fewer rows of cells in these structures but rather to the fact that the cells are smaller and narrower. Like those of *M. cerifera* the medullary-ray cells of the xylem have their walls more or less lignified and serve for the storage of starch. The primary medullary-rays are mostly 1-5 cells wide, the secondary 1-2 and 1-3 cells wide.

8. The pith is composed of active polygonal to rounded cells, whose walls are lignified. They serve for the storage of starch. Some frequently contain gummy-lignin.

Subterranean branch of *Myrica Carolinensis*, Miller

There are fewer layers of cork formed than in the aerial stem. The cells of the cortex are somewhat larger, more rounded and contain more starch. The intercellular air-spaces between cortical parenchyma cells are larger and devoid of calcium oxalate crystals. The sclerenchyme elements in the cortex are smaller and fewer than in the above ground stem. The pericycle differs from that of the aerial stem in having widely separated small groups of narrow sclerenchyma elements.

The phloem region is composed of wider elements than exist in the similar region of the above ground stem. It is traversed by broader medullary-rays which are from 1-4 to 1-5, rarely 1-6, rows of cells wide (primary medullary-rays) or from 1-2 to 1-3 rows of cells wide (secondary medullary-rays). Bast fibres are present in both proto- and metaphloem regions, in small groups, which are surrounded by crystal fibres containing monoclinic prisms of calcium oxalate. The pitted tracheae of the xylem are fewer than in the aerial stem and about twice as broad. The largest have a maximum breadth of 81.56 μ. The xylem medullary-rays are numerous and have cells considerably broader than those of the aerial stem. The primary medullary-rays are mostly 1-5, rarely 1-6, rows of cells wide. The secondary medullary-rays are from 1-2 to 1-3 rows of cells in breadth. The autumn wood is similar to that of the aerial stem. The pith region is uniformly wider, its cells somewhat smaller, and with thinner walls than the corresponding area of the aerial stem. Many of its cells, like those of the cortex, contain starch and gummy lignin.

Aerial stem of *Myrica Macfarlanei*, Youngken
(*M. cerifera* x *M. Carolinensis*)

The aerial stem of the hybrid resembles that of *M. cerifera* in the following structural details:—(a) a continuous sclerenchyme zone in the pericycle, (b) the tendency of the phloem masses to become arched in their outer portions due to the broadening out of the medullary rays at their extremities, (c) the presence of many bast fibres accompanied by crystal fibres as well as stone cells in the phloem, (d) the uniformity in distribution of the tracheae. It resembles the aerial stem of *M. Carolinensis* in the size and relative number of tracheae found in the protoxylem.

It differs from both parents as follows—The autumnal wood is intermedate in thickness; the pitted tracheae are fewer than in *M. cerifera*, more numerous than in *M. Carolinensis*; the mean diameter of the tracheae is intermediate between that of both parents (Plate 87, Fig. 20). The primary medullary rays are 1-3, rarely 1-4, rows of cells wide; the secondary, 1-2 rows in width. Other stem structures common to both parents are likewise seen in the hybrid.

Subterranean branch of *Myrica Macfarlanei*, Youngken

The rhizome of the hybrid between *M. cerifera* and *M. Carolinensis* shows the following histological peculiarities:—Compared with the aerial stem of the same age, it shows fewer cork layers and a broader cortex. The cells of the cortex are generally larger, less angular, and contain more starch grains. The bast fibres of the phloem are about twice as numerous and in larger groups. The tracheae are fewer and generally broader, while the woody fibres are more numerous and more lignified. The pith is narrower; its elements smaller and more lignified. The primary medullary-rays are broader and frequently show 1-5, less often 1-6, rows of cells in width. The secondary medullary-rays vary in width from 1-2 to 1-3 rows of cells. Cortical parenchyme, phloem, and tracheal elements with a gummy lignin content are more frequent than in the above ground stem. Compared with rhizomes of its parents, it shows intermediate characters in respect to the number and mean diameter of the tracheae and the woody fibres.

Aerial Stem of *Myrica Gale*, L

(Plate 87. Fig. 23.)

In passing from periphery toward the centre, the following histological peculiarities are seen:—

1. A cork zone of unevenly stained brick-shaped cells whose walls are partly suberized and partly lignified. The lignification occurs on the inner tangential walls.

2. A phellogen of thin walled tangentially elongated cells.

3. A comparatively narrow cortex composed of cells some of which are thick walled and contain gummy lignin, some thin walled, containing starch while others are rich in rosette, monoclinic prism, or crystals and forms of calcium oxalate. The intercellular air-spaces of this region are small to medium and angular. Many contain several crystals of calcium oxalate. The cortex is entirely devoid of sclerenchyme elements.

4. A pericycle which contains an interrupted zone of sclerenchyme elements.

5. A very narrow phloem, even in old stems, which is devoid of bast fibres but contains numerous sieve tubes and phloem parenchyme cells. Through this region course numerous medullary-rays which broaden out toward their extremities and arch in many phloem masses.

6. A cambium of one layer of meristematic cells with thin walls.

7. A xylem composed of numerous narrow compactly arranged wood wedges separated by medullary-rays which are mostly 1 cell wide (second-

ary med. rays); a few (primary med. rays) vary from 1-2 to 1-4 rows of cells in width. The cells of these medullary-rays exhibit marked protoplasm connections which are best seen in a radial longitudinal section. Their walls are lignified. The wood wedges are composed mostly of tracheids with bordered pits. The tracheae are generally pitted and mostly found in the spring wood. Like those of the other stems treated, they show barred septa. They are arranged radially and are more or less polygonal in transverse section. Some of them show a bacterioid content. Spiral tracheae, as in the other stems, are found in the protoxylem.

8. The pith, as noted by Gris,[12] is composed of thick walled cells which are mostly functionally active. Some of them contain gummy lignin.

Subterranean Branch of *Myrica Gale*, L

The subterranean branch of this plant differs microscopically from the above ground stem in the following particulars:—The cork zone is narrower. The cells of the cortex are somewhat larger and show fewer crystals of calcium oxalate. There are more tracheids and fewer spiral ducts in the protoxylem. The pitted tracheae with bacterioid contents are more numerous. The tracheae tend to become larger.

Aerial Stem of *Comptonia Asplenifolia*, (L.) Aiton

Passing from exterior toward the centre one notes the following structures:—

1. A cork consisting of several layers of irregularly brick-shaped cells which stain unevenly with safranin and methyl green. Many of them have their inner tangential walls more or less lignified.

2. A cork cambium (phellogen) of thin walled meristematic cells.

3. A cortex of tangentially elongated parenchyme cells, some of which contain starch, others gummy lignin, tannin or crystals of calcium oxalate of rosette or tabular form.

4. A pericycle displaying an almost continuous zone of sclerenchyme elements.

5. A phloem containing numerous more or less oval shaped groups of bast fibres arranged in interrupted circles and separated from one another by the soft bast elements. (Plate 87. Fig. 22.) Each group of bast fibres is surrounded by a number of crystal fibres whose cells contain monoclinic prisms of calcium oxalate. Considerable tannin is evidenced by the use of ferric chloride test solution. The phloem medullary rays are 1-5 rows of cells wide.

6. A cambium forming a very wavy zone of tangentially dividing meristematic cells.

7. A xylem which is quite porous and composed of numerous wood wedges separated by medullary-rays, for the most part 1-2 rows of cells wide. The tracheae of the metaxylem show broad transverse pits in their walls as well as barred septa and *Actinomyces* forms. The woody fibres are extremely lignified especially in the autumn wood. The medullary-ray cells show many prominent protoplasmic connections.

8. The pith, like that of *M. Gale*, is irregularly lobed. It is composed of parenchyma cells which become lignified very early. These cells are of variable size and appear mostly rounded in transverse section. Many of them contain gummy lignin, while some possess rosette crystals of calcium oxalate.

Subterranean Branch of *Comptonia Asplenifolia*, (L.) Aiton

The microscopical characteristics of an underground branch of this plant are for the most part similar to those of an aerial stem. The important differences are a diminution in the number of cork layers, the tendency of the medullary ray cells to become broader, the presence of more cells containing gummy lignin and a slight increase in the diameter of the tracheae.

Comparative Morphology of the Root Tubercles

Within the past thirty years various investigations have been carried on by Brunchorst, Möller, Shibata, Chevalier, Harshberger and Arzberger in respect to an endophytic organism living in the tissues of the Myricas and forming tubercles.

Brunchorst[13] was the first to mention the tubercles on Myrica Gale and named the fungus producing them—*Frankia Subtilis*—because he considered this organism similar to that in the tubercles of *Alnus*.

Möller[14] later found the organism to differ considerably from that infesting *Alnus* and named it—*Frankia Brunchorstii*.

Shibata[15] investigated the tubercles found on *Myrica rubra*, his observations being on both fresh and preserved material. He described the morphology of the tubercle, showing that the fungus confines itself to a ring of from 1-3 layers of cells beneath the cork thus differing from the condition found in *Alnus*. He also pointed out that infection takes place acropetally by means of fungal threads. He traced these threads into the already differentiated meristematic cells where they grew rapidly to form a dense thready reticulum, then branched into radiate threads whose free ends became swollen in clavate fashion. He assigned to the fungus a position in the genus *Actinomyces*.

Chevalier, (7 p. 124-139) examined the tubercles on the roots of *Myrica Gale* (*Gale palustris*), *Myrica cerifera*, *Myrica Carolinensis* (*M. Pennsylvanica*), and *Myrica sapida* var *longifolia*. He found them on main roots, adventitious roots of subterranean branches, and on subterranean stems. He described at length their general gross structure and histology, the occurrence of gummy lignin in the cells attacked, and called the infesting organism, *Frankia Brunchorstii*, previously observed by Möller.

Harshberger[16] observed the tubercles on the adventitious roots of *Myrica cerifera*. He studied the structure of the mature tubercles from dry material only which had been boiled in water and afterwards treated with alcohol. He called the tubercles "mycodomatia" and claimed for the infesting fungus a position closely related to the Oömycetes.

Arzberger[17] investigated the root tubercles of *Myrica cerifera*, *Myrica Gale*, and *Comptonia asplenifolia* (*Myrica asplenifolia*) and stated like Harshberger that these structures appear on adventitious roots which grow out from the lower part of the stem or from branches or stems which have been covered over with leaf mold or soil for several years. He described the morphology and cytology of the tubercles but his illustrations do not show the true nature of the radiating clavate branches. He favored the opinion of Shibata in placing the fungus in the genus *Actinomyces*.

During the last three years the writer has collected and examined abundant tubercle material of *M. cerifera*, *M. Carolinensis*, *M. Macfarlanei*, Youngken, (*M. cerifera*, *M. Carolinensis*) Youngken and *Comptonia asplenifolia* at Palermo and Tuckahoe, N. J.; of *M. Carolinensis* and *Comptonia asplenifolia* at Clementon and Albion, N. J.; of *Comptonia asplenifolia* near Mainville, Pa.; and of *M. cerifera*, *M. Carolinensis*, and *M. Macfarlanei* at Wildwood and Rio Grande, N. J. He has raised *M. cerifera* seedlings bearing tubercles from seeds which he planted in sandy soil in the University of Pennsylvania greenhouse. He has furthermore examined tubercle material of *M. Gale* collected by Dr. John M. Macfarlane along Trefethan Bay in Chebeague Island of Casco Bay and on the southeastern part of Peak's Island in Casco Bay, Maine.

Methods

Material from many plants of each species was macroscopically and microscopically examined both in its fresh and preserved condition. All of the preserved material was fixed in weak, medium and strong Flemming's on the ground immediately after the position and nature of the tubercles on the plants had been ascertained. Samples of each lot were then dehydrated in gradually increasing strengths of alcohol, cleared in cedar oil and xylol and imbedded in paraffine. Transverse, tangential, longitudinal radial, and longitudinal tangential sections were then cut 6-10 microns thick and subsequently stained in several ways. The best results were obtained with the Methylene Blue and Acid Fuchsin combination, although satisfactory results were also obtained with a combination of Safranin and Gentian Violet.

The writer employed the following technique in isolating the endophyte which produces the tubercles on *M. cerifera*, *M. Carolinensis*, *M. Macfarlanei*, *M. Gale*, *Comptonia asplenifolia* and probably most, if not all, of these lesions on other plants of the Myricaceae.

A tubercle cluster from a root of one of the seedlings grown in the University of Pennsylvania greenhouse was washed thoroughly with clean water to remove all traces of adhering soil. It was then introduced into a test tube containing 1:1000 corrosive sublimate solution for 20 seconds in order to destroy any surface organisms. From this it was transferred with sterile forceps to a test tube containing distilled water which had previously been sterilized in the autoclave. Into this was introduced a sterile scalpel and two of the tubercles were cut into small fragments. These fragments were next transferred to 5 tubes of sterile slant agar by means of a sterile platinum loop. The tubes containing the culture were then stored in a dark closet at ordinary room temperature for several weeks. All 5 cultures when examined revealed the presence of *Actinomyces* rosettes, non septate thin filaments, and rods of different sizes as well as coccus forms, all of which stained well by Gram's method. The coccus forms are probably for the most part products of the degeneration of the above filament.* Jordan supports this view in regard to similar forms of *Actinomyces* found in cattle, sheep, hogs and man. The *Actinomyces* rosettes were found to be present in the depth of the agar. This shows the anaerobic nature of the organism.

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*From two of the above cultures. the writer has recently successfully grown pure sub-cultures on coagulated horse serum in sealed tubes, kept at the temperature of 37.5° C.

Five seedlings of *M. cerifera*, which the writer had previously grown from seed in the University of Pennsylvania greenhouse, were then removed from the soil and their root systems loosened from adhering sand by gently washing in clean water. With the aid of Mr. Lambert, of the University Gardens, who, like the writer took special care to insure against sources of infection by other organisms, the root systems were one by one quickly dipped into 1:1000 corrosive sublimate solution and then washed in sterile distilled water. While Mr. Lambert, with sterile hands, held each seedling so treated, the writer, by means of a long needle, previously sterilized by passing through the bunsen flame, removed a small portion of the *Actinomyces* culture from one of the tubes and pricked it into the root of 4 seedlings, marking the place of inoculation by tying a sterilized piece of cord just above the puncture. The last seedling was treated similarly to the first four, with the exception that it was merely pricked with a sterile needle. This served as a control. Each seedling was then planted in a sterile pot containing sterile sand. Both pot and sand were previously sterilized in the hot air oven at a temperature of 210° C. for 8 hours. The potted seedlings were then placed in a special case in the greenhouse and daily watered with sterile Knop's solution. At the expiration of 9 weeks the seedlings were carefully removed, washed in clean water and their roots examined for the presence of tubercles. These were found in a primitive state at the points of inoculation on all but two including the control, which was pricked with a sterile needle only Thin hand sections of one of the tubercles revealed the presence of *Actinomyces* in the same condition as observed in the cells of the tubercles of the *M. cerifera* seedling, as well as of the tubercles on the other species above noted. The appearance of the infesting *Actinomyces* within the cells of the host plants will be treated under the caption dealing with the histology of the tubercles.

Gross Structure of Tubercles

The writer has found tubercles on the *M. cerifera*, *M. Carolinensis*, and *M. Macfarlanei* seedling primary roots of 5 to 6 months' growth, and from thence onward on the secondary roots inserted on the hypocotyl axis, on nearly all the adventitious roots of subterranean branches and on the subterranean branches of *M. cerifera*, *M. Carolinensis*, *M. Gale*, *M. Macfarlanei*, and *Comptonia asplenifolia*.

The tubercles occur either singly, as is frequently the case on subterranean branches, in small groups the size of a pea, or in larger coralloid loose or compact clusters which frequently attain the size of a large black walnut. Each tubercle is a short cylindrical blunt-ended root like structure which branches di- or trichotomously after attaining a certain length. The branches frequently rebranch at their tips, which grow out into long thread-like structures from 1-3 cm. in length that may also branch and become entwined about the roots of other plants. The maximum length of a tubercle is 5 mm. The average length of the branches being from 2-3 mm. The color of the youngest tubercles is a pinkish gray-brown. As the tubercles become older their color changes to brown, dark-brown and even black.

Histology

The tubercles when studied microscopically exhibit the following structural detail:

A cork, constituted of from 2 to 4 layers of suberous cells, whose outer ones are dead, filled with gummy lignin, and in the process of exfoliation, forms the external bounding layer. The cork tissue is derived from the outer layer of pericambium of the host root which functions as a phellogen during the development of the tubercle. Beneath the cork lies a very broad cortex, which, instead of being formed as in normal roots of 5-12 layers of cells separated by large intercellular air spaces, is constituted of 15-24 layers of very closely united parenchyma cells. The outer 3-5 layers of this region are composed of rounded to tangentially elongated cells, some of which contain starch grains, others tannin, a few gummy lignin. Underneath this lies a zone usually 2 to 3 cells broad of radially elongated cells and a few smaller rounded cells which are separated by small air-spaces. The radially elongated cells are hypertrophied and contain the *Actinomyces* parasite (Plate 88, Fig. 24), which may or may not be enveloped by gummy lignin. Many of the abutting smaller cells are rich in tannin and show no evidence of the parasite. Beneath this zone of infested cells is found a usually broader zone of smaller isodiametric cells intermingled with a few oblong cells. In *M. Carolinensis* as noted by Chevalier, in *M. Macfarlanei*, *M. Gale* and *Comptonia asplenifolia* as noted by the writer, it frequently happens that other cells scattered without order throughout the cortex are also infested by *Actinomyces*. These like those of the infested radially elongated zone are also hypertrophied. All of the infested cells are united by means of *Actinomyces* threads which run through the cell walls from cell to cell as well as the intercellular air-spaces. The endodermis or innermost layer of the cortex is composed of small oval thick-walled cells which contain a yellowish-brown substance (gummy lignin). The walls of these cells become suberized very early. Underneath the endodermis is found the vascular cylinder, which is quite reduced in size as compared with that of the normal root. In the young tubercle it is constituted of a a radial tetrarch fibrovascular bundle which surrounds a small pith. The phloem elements of the bundle become inactive very early. The xylem is composed mostly of wood fibres intermingled with a few tracheae. Secondary development is of very short duration.

In the younger tubercles the vascular cylinder extends only part way into the apex, while in older ones the cylinder, with some cortical parenchyma cells surrounding it, grows out into a slender thread from which lateral branches are then sent off.

Actinomyces living in the tubercles is best observed in its various relations, in a radial longitudinal section. There the youngest stages may be observed in the meristematic region of the apex, while the older stages may be traced back toward the base of the tubercle. As observed by Shibata in *M. rubra* tubercles, the writer has likewise noted in the case of the tubercles of *M. cerifera*, *M. Carolinensis*, *M. Macfarlanei*, *M. Gale* and *Comptonia asplenifolia* that the differentiation of the ring of infested cells starts in the meristem near the growing apex, thus indicating that infection takes place acropetally. Since tubercles are found on the seedling roots of 5-6 months' growth, it would indicate that infection takes place very early in the life of the young seedlings.

These infested meristematic cells become radially elongated and are found to contain several extremely fine non septate and branched thread-like structures. The *Actinomyces* (*Streptothrix*) threads extend through the transverse and longitudinal walls of these cells aided evidently by the secretion of a ferment which dissolves the cell wall in the line of the organism's progress. They then invade neighboring cells where they run between the starch grains toward the nucleus around which the organism seems to derive its greatest benefit. Shortly after the appearance of parasite threads within the invaded cells, the starch grains become dissolved and in this form are appropriated as food by the organism. The nucleus becomes hypertrophied and finally perishes. The endophyte by this time has grown very rapidly into a dense thready reticulum. Gummy lignin appears at first of a clear yellow color but later becoming yellowish brown. The dense thready web of the organism sends out clusters of radial thread branches forming an *Actinomyces* rosette, which in many cells completely fills up the whole cell lumen. These threads frequently become club shaped at their extremities. Some of the threads after piercing through the wall of a cell develop clavate ends.

In due course of time, as is evidenced in many older infested cells, the fungal threads become shrunken together and impregnated with gummy lignin forming a good sized lump of degenerating material within the cell, which remains connected with similar lumps, or mycelial webs of adjacent cells by means of threads which penetrate the cell wall. Some of the cells containing the rosettes also show coccus-like forms, while other cells, especially in the older basal portion of the tubercle, are almost completely filled with these. These cocci are probably products of the disintegration of the filament. They may be involution forms of the *Actinomyces* organism which appear in cells whose contents are poorly adapted to the trophic needs of the endophyte. Their presence in such numbers on artificial culture media would support this hypothesis. While *Actinomyces* is the primary infecting agent responsible for the tubercles on *Comptonia asplenifolia*, there frequently later appears in the cells and intercellular air-spaces of some of the tubercles a mycelium producing fungus with unseptate hyphae belonging probably to the Oömycetes as Harshberger suggested. The hyphae of this fungus are several times as thick as those of *Actinomyces*. They penetrate through the cell walls of the tubercle, passing from cell to cell and often coil up into a mycelial mass in many of the cells invaded (Plate 88, Fig. 27).

Since *Actinomyces* is frequently a virulent pathogenic organism in cattle and other domestic animals up to man, because the swellings it produces on plants are analagous to those on animals, since the forms of the organism as shown by Jordan[18] in the infested lesions of animals are similar to those which the writer has described in the lesions of *Myrica*, and since the cultural characteristics of the organism isolated from the lesions of animals by Wright,[19] Wolff and Israel[20] are in many respects similar to those isolated from the Myricas and described by the writer, he would regard the organism as a parasite and suggest its possible pathogenic relation to such animals.

The *Actinomyces* not only confines itself to the cortex of the tubercular roots, it later works its way into the tracheae of these structures, passes into the pitted vessels of the main roots, thence into those of the stems, and, conveyed by the transpiration stream gradually upward is carried through the axes of catkins so as finally to reach the flowers, bracts and fruits. In these it confines its existence to the parts corresponding to the mediocortex of the root tubercles, namely the mesophyll and outer mesocarp regions respectively.

The writer having isolated the organism in pure culture from the lesion produced by it on the seedling tubercles hereby assigns to it the name *Actinomyces myricarum*.

COMPARATIVE MORPHOLOGY OF THE LEAVES

Gross Structure

Myrica cerifera, L.

The leaves of this plant are simple, alternate, exstipulate, inserted in a 2/5 spiral, pinnate-reticulate in venation, fragrant with a balsamic resinous odor, coriaceous, evergreen and appearing toward the ends of the branches which bear the flower buds of the following season about the middle of May and persisting until the flower buds begin to open, when they gradually fall, as the new leaf buds expand without assuming a copper-red color. They are lanceolate-cuneate or oblong lanceolate, (Plate 83, Fig. 6) varying from 30-100 mm. in length and 5-15 mm. in width, often drawn out toward both extremities, acute, mucronate, or less often obtuse, or sharply notched at the apex, long cuneiform at the base and decurrent on the short stout petiole. Their margin is thickened, incurved beneath, very slightly eroded, entire or showing one to several teeth in the anterior half or third. The upper surface of the lamina is of a shining dark-green color and exhibits numerous crowded pits, some of which contain orange-red and others golden-yellow glandular hairs. Simple hairs are also present on the depressed midrib and along the margin in the young condition of the leaves, but disappear as the leaves become older These are fewer than on *M. Carolinensis* or *M. Macfarlanei*, leaves. The lower surface of the lamina is of a yellowish-green color showing fewer simple hairs than *M. Carolinensis* in the young condition of the leaves. The simple hairs are entirely absent or very rare on this surface of older ones. Orange-red and golden-yellow glandular hairs are very numerous as on upper epidermis. Both midrib and lateral veins, as well as many branches of the lateral veins are very prominent, the lateral veins being inserted on the midrib at an angle of 45-60°.

Myrica Carolinensis, Miller

Leaves simple, alternate, exstipulate, inserted in a 2/5 spiral, pinnate-reticulate in venation, fragrant, membranous, deciduous, beginning to appear toward the ends of the branches which bear the flower buds of the following season the latter part of April (the writer has observed many leaves expanded at Clementon, N. J. on April 23, 1915) and in full foliage by the first or second week in May. They remain green all summer, assume a greenish-brown hue on an extensive scale in October and November and usually have completely fallen by the middle of

December. An exception to this rule was noted by the writer at Clementon, N. J. where on February 14, 1915, in a valley protected by tall pines and harboring abundant underbush, the young plants retained many of their leaves, but the majority were partly discolored. They are usually elliptic-obovate, varying from 30 to 105 mm. in length and from 18 to 45 mm. in width, the maximum size being attained on the sapling shoots, rounded at the summit and shortly apiculated, feebly attenuated at the base; the most with margin entire, very hairy, feebly incurved below, the others presenting in their anterior half small to large thickened, rounded crenations, each terminated ordinarily by a very small point. The petiole shows a few golden-yellow glandular hairs but covered with white simple hairs on both faces. The upper surface of the lamina is of a bright green color, more or less shining, covered in the adult state with short simple hairs amongst which are scattered golden-yellow glandular hairs, never very numerous. The lower surface is dull green and shining in color, finely reticulated, showing numerous simple white hairs and golden-yellow glandular hairs. The mid-rib and lateral veins while visible on the upper surface, are more prominent on this surface.

Myrica Macfarlanei Youngken. (*M. cerifera x M. Carolinensis*)

The leaves of this hybrid between *M. cerifera* and *M. Carolinensis* show several striking macroscopic characters, which are intermediate between those of its parents. For instance, they vary from lanceolate-cuneate to elliptic-obovate in shape, many of them being a mean between these two forms (Plate 85. Fig. 13). In duration they are semi-evergreen, and usually fall during February-March, by which time they have often assumed a slight coppery tint. In size, they vary from 25-58 mm. in length and from 8-20 mm. in width. They have numerous orange-red and golden-yellow glands on their lower surface with merely a few of each of these on their upper surface. Simple hairs are found on both surfaces and margin, as on the leaves of *Myrica Carolinensis*, but relatively fewer in number. The texture of the leaves is sub-coriaceous. In color the leaves have blended the color characteristics of both parents. The margin is more incurved beneath than *M. Carolinensis*, less than *M. cerifera*.

Myrica Gale, L

The leaves of this plant are simple, alternate, exstipulate, inserted in a 2/5 spiral, pinnately and reticulately veined, subcoriaceous, deciduous, varying in length from 25 to 90 mm., in width from 8-30 mm., oblong, lanceolate or oboval cuneiform in shape, longly attenuated, cuneiform at the base; margin entire along the 2/3 or 3/4 of their length, somewhat thickened and hairy, slightly incurved below, showing ordinarily toward the summit from 3 to 5 pairs of small serrations, acute mucronate, or obtuse at the apex. (Plate 84. Fig. 8 and 9). The petiole is 1-3 mm. long with its superior surface plane and thin as well as glandular hairy. The upper surface of the lamina is dark green, slightly thin hairy, wrinkled, covered with depressed reticulations and showing a scattering of golden yellow glands which are fewer than on the lower surface; mid-rib depressed, secondary nerves non apparent. The lower surface is of a pale green color. It is covered with thin hairs and numerous golden-yellow glands. The mid-rib on this surface is projecting and tomentose; the secondary nerves are inserted on it at an angle of 45-60°.

Myrica inodora, Bartram

Leaves simple, alternate, exstipulate, inserted in a 2/5 spiral, pinnately and reticulately veined, coriaceous, evergreen, oblong-obovate or occasionally ovate, obtuse or sometimes pointed and occasionally apiculate at the apex, narrowed at the base, decurrent on the short stout petioles, the margin thickened, entire or rarely obscurely toothed toward the apex, varying in length from 30-100 mm. and in width from 17-38 mm. When they unfold they are covered with pale yellow glands. The upper surface is glabrous; lustrous dark green, showing a somewhat depressed glandular mid-rib and lateral veins. The lower surface shows its mid-rib veins more prominent. The mid-rib is often slightly puberulous. Sargent states that the leaves of this plant begin to fall in May and disappear from the branches before mid-summer. They differ from the fragrant leaves of the other eastern species in being inodorous.

Myrica cerifera var. *pumila*, Michaux

Leaves simple, alternate, exstipulate, sessile or with a petiole 1-2 mm. long, inserted in a 2/5 spiral, pinnate-reticulate in venation, coriaceous, evergreen, oblanceolate, varying in length from 20-45 mm., and in width from 5-10 mm., acute or mucronate at the apex, cuneately narrowed at the base. The margin is revolute, slightly thickened and incurved beneath, commonly saw-toothed in its upper 1/3. The petiole when present is the shortest of any of the eastern species. In other respects the herbarium material studied closely resembles the leaves of *M. cerifera.*

Comptonia asplenifolia, L. Aiton

The leaves of this plant are alternate, inserted in a 2/5 spiral, petiolate, stipulate, pinnately-veined, distinctly pinnatifid, showing 4-15 pairs of sub-reniform pinnules measuring 2-14 mm. long and 2-10 mm. wide, separated from each other by a sinus extending almost to the mid-rib, membranous, deciduous, with membranous caducous stipules (Plate 82. Fig. 5). They vary in shape from lanceolate to elliptic-ovate and in size from 10-138 mm. long to from 2-22 mm. wide. Each lobe, as previously shown by Chevalier, receives ordinarily two secondary nerves and two demi nerves arising from a thread corresponding to the indentation, which thread, arriving at the sinus, divides into two branches distributed to two adjacent lobes. More often the sinus extends to within 1 or 2 mm. from the mid-rib. The small secondary nerve which normally divides into two has not the time to bifurcate and is usually thrown into the superior lobe. The result is that all the lobes have 3, 4 or 5 secondary nerves of approximately equal importance. Occasionally 2 lobes completely fuse to form an auricle with a maximum length of 12 mm. This receives a double number of nerves. The stipules are auriculate or semi saggitate, up to 13 mm. long, gibbous at their base, acuminate at their summit, hairy around their margin. The petiole is sub-cylindric, thin hairy and showing a sparse covering of glands (contrary to Chevalier's statement that glands are absent). The pinnules are entire or showing occasionally a tooth, with a thin hairy margin, which is not incurved below. The upper surface is dark green tomentose in young leaves, becoming nearly glabrous as the leaves attain maturity.

The lower surface is light green, thin hairy in the young state of the leaf but dropping as the leaf ages. Both surfaces show shining yellow glands, not very numerous. These are somewhat more frequent on the lower than on the upper surface.

Histology

Myrica cerifera, L

The upper epidermis consists of a single layer of cells whose outer walls are strongly cuticularized and have rectilinear or very slightly curvilinear vertical walls. Chevalier (7, p. 113) errs in stating that *Comptonia* is the only species whose upper epidermis shows cells with walls slightly curvilinear. Over the veins the cells are rectangular and elongated in the direction of the leaf bundles. The mean dimensions of these cells are $23.20\,\mu \times 15.20\,\mu$. The cuticle is punctated by very small pearls within which, according to Chevalier, (7, p. 113) are associated fine granules of wax. The summit of each epidermal cell forms a rounded projection which together with others give a rough aspect to the surface. The bottom and transverse walls remain thin. The surface of this epidermis contains numerous crypts not any larger than those of the lower epidermis. Some of these crypts contain a stalked golden-yellow balloon-shaped gland, while others possess a stalked reddish-brown bowl-shaped gland which becomes black as the leaf ages. The stalk of the balloon gland is 4-7 cells high and two rows of cells in width. The gland is unicellular and contains oil in its young state which resinifies as the gland becomes older. The stalk of the bowl-shaped gland ordinarily varies from 7-8 cells in height and is 4 cells wide on the mature adult leaf. The head or gland is multicellular and contains resin. Very few short unicellular simple trichomes are found. These when present are sclerified at their bases. Scattered here and there on the surface and in the middle of normal cells are isolated refractile elements which represent the bases of these unicellular simple hairs which have broken down on the level with the epidermis and are completely sclerified. Stomata are absent on this epidermis. The mesophyll shows palisade and spongy parenchyma differentiation. The palisade zone is 4-5 cells broad and is composed of columnar elements usually about twice as long as wide. Some of these elements contain rosette crystals of calcium oxalate. The spongy parenchyma region consists of chains of oval to irregular shaped cells surrounding large intercellular air spaces. The fibro-vascular bundle of the mid-rib, as well as smaller vein bundles, run through this region. Each is connected with the upper and lower epidermis by means of hypodermal cells, many of which are enlarged and contain a rosette of calcium oxalate. The fibro-vascular bundle of the mid-rib is reinforced above and below by an arc of sclerenchyme. The xylem of this bundle is quite broad and shows medullary rays 1-cell wide, which radiate like a fan. The ducts show spiral thickenings. The phloem forms a thick semilunar band below the xylem.

The lower epidermis consists of polygonal cells whose outer walls are much less cuticularized than those of the upper epidermis. Like those of the upper epidermis they contain a brownish content. Their vertical walls are curvilinear. The mean dimensions of these cells are

24.28 μ x 12.14 μ. Stomata are scattered amongst the lower epidermal cells without order. Each in the mature adult leaf is surrounded by 6-8 neighboring cells. Crypts are more numerous than on the upper epidermis. These contain similar glands to those found on the upper surface. Very few 1-celled trichomes or their bases are ordinarily found.

Myrica Carolinensis, Miller.

The upper epidermis of this leaf consists of polygonal cells, whose outer walls are covered by a thin cuticle. The vertical walls are rectilinear. Over the veins the cells are rectangular and elongated in the direction of the bundles. The cells are filled with a brownish substance as in *M. cerifera* and *M. Macfarlanei*. Their mean dimensions are 29.13 μ x 17 μ. Scattered over this epidermis are numerous simple unicellular trichomes with sclerotic bases, as well as the bases of sclerotic hairs, each of which is surrounded by several rows of cells arranged in radial fashion. Very few or no golden yellow glandular trichomes are found. These are more frequently present than absent. Each consists of a stalk and a gland or head. The stalk is 4-7 cells in length and two rows of cells wide. The gland is balloon-shaped. The glandular trichomes are inserted into the epidermis at the bottom of crypts which are less numerous on the upper than on the lower surface.

The mesophyll, as in the leaves of *M. cerifera*, shows dorso-ventral differentiation into palisade and spongy parenchyma regions. The palisade zone consists of 2-3 layers of loosely arranged columnar shaped cells, some of which contain rosettes or monoclinic prisms of calcium oxalate. The spongy parenchyma region comprises a network of oval to irregular shaped cells arranged in chain fashion and surrounding large intercellular air spaces. Some of these cells contain crystals of calcium oxalate. The fibro-vascular bundle of the mid-rib and smaller veins course through this region. The stronger veins are united to the upper epidermis by means of hypodermal elements which are collenchymatic. Other veins are united to both epidermises by hypodermal cells containing crystals of calcium oxalate. The fibro-vascular bundle of the midrib is surrounded above and below by an arc of sclerenchyme, the upper arc as in *M. cerifera* being the more strongly developed of the two.

The lower epidermis consists of wavy walled cells which have the mean dimensions of 28.02 μ x 14.4 μ. Numerous stomata occur on this epidermis. These are surrounded by 5-6-7 neighboring cells. Crypts are numerous and scattered without order over the lower surface. Each contains a golden-yellow balloon-shaped gland subtended by a stalk two rows of cells wide and 4-7 cells high. The base of each balloon-shaped gland stalk is surrounded by 16 somewhat elongated cells arranged in radial fashion. Simple unicellular trichomes also occur in large numbers on this surface and along the margin. The base of each is sclerified and elevated in papillar fashion above the epidermis. No bowl-shaped glands occur on either surface of the leaf. The average width of the lamina outside of the mid-rib is 168.96 μ.

Myrica Macfarlanei, Youngken.

The microscopic characteristics of the leaf of this hybrid are intermediate between those of its parents. For instance:—the upper epidermis is composed of cells whose vertical walls are more curvilinear than *M. cerifera*, less curvilinear than *M. Carolinensis.* Their mean dimensions in surface view are 26.7 μ x 16.89 μ. Their outer walls are less projecting than those of *M. cerifera*, more so than *M. Carolinensis.* The cuticle is thinner than that of *M. cerifera*, thicker than that of *M. Carolinensis.* The palisade region of the mesophyll is 3-4 layers wide and so is intermeditate between this region in *M. cerifera* and *M. Carolinensis* (*vide supra*). The lower epidermis consists of cells whose vertical walls are more curvilinear than those of *M. cerifera*, less curvilinear than those of *M. Carolinensis.* The mean dimensions of these cells in surface view are 26.46 μ x13.59 μ which is likewise an intermediate character. The stomata are fewer in number than on *M. Carolinensis*, more numerous than on *M. cerifera.* The upper surface shows a scattering of orange-red bowl-shaped glands and golden-yellow balloon-shaped glands, or sometimes the latter only. The lower epidermis shows numerous orange-red bowl-shaped and golden-yellow balloon-shaped glands, both of which are often fewer than on the similar epidermis of *M. cerifera.* The tendency for the head of the bowl-shaped gland in the hybrid to become saucer-shaped is very striking. Simple unicellular trichomes and the sclerotic bases of these are common on both lower and upper epidermis, but intermediate in number between those on the leaves of the parents. Finally, the average thickness of the lamina of the hybrid outside of the mid-rib is 201.6 μ while that of *M. cerifera* is 268.8 μ and of *M. Carolinensis*, 168.96 μ.

Myrica Gale, L

The upper epidermis is composed of a layer of polygonal cells with rectilinear to very slightly curvilinear external walls. It shows here and there stomata which are fewer than on the lower epidermis. The cuticle of this epidermis is somewhat thicker than that of the lower epidermis. Many of the epidermal elements contain brownish contents. In some the contents appear granular. Numerous bases of sclerotic unicellular hairs are scattered here and there over the surface. A few sclerotic hairs are found and generally occur above the mid-rib and the veins. Each of these is somewhat elevated and surrounded by usually radially arranged cells. Crypts, containing stalked yellowish glandular hairs are numerous. These are generally deeper than those of the lower epidermis. The mesophyll shows a differentiation into a broad upper palisade zone, a middle spongy parenchyme region and a narrow lower palisade layer. The upper palisade zone consists of three layers of columnar shaped cells with variously stained contents. Some of them contain crystals of calcium oxalate which may be in the form of rosettes, monoclinic prisms or crystal sand. Others contain one to several radiating structures which suggest the *Actinomyces* rosette. The spongy parenchyme region is composed of small celled elements which are irregularly shaped and surround intercellular air spaces. The lower palisade layer is incompletely differentiated and consists of columnar as well as irregular shaped cells, which are loosely arranged. Some of these contain crystals of calcium oxalate. The fibrovascular bundles of the mid-rib and veins run through the spongy parenchyma zone. Those of the stronger veins, as well as that of the mid-rib, are connected with both lower and upper epidermis by hypodermal elements. Those above the bundles are usually lignified. Many of the non-lignified hypodermal elements contain rosettes of calcium oxalate. The vascular bundle of the smaller veins is connected with both the upper and lower epidermis by special layers of elongated cells with wide lumina. The lower epidermis is less cutinized than the upper and consists of cells which for the most part have their outer walls arched in papillose fashion giving to this epidermis a roughened aspect. Numerous crypts containing yellow stalked balloon-shaped glandular trichomes are found, but these appear broader and shallower than those of the upper epidermis. Stomata are also present but more numerous than on the upper surface. They are covered in by neighboring cells. The yellow balloon-shaped trichomes consist of a stalk or pedicel supporting a balloon-shaped head. The stalk is usually 3 cells high, the basal layer alone consisting of 2 cells. The head or gland contains oil which resinifies as the leaf ages. Simple unicellular sclerotic trichomes as well as the bases of these are also found, but in larger numbers than on the upper epidermis. Occasionally a 3-4 celled uniseriate glandular hair is found which has its terminal cell or one of its middle cells specialized as a gland and containing oil. Isolated epidermal cells of the ordinary kind often become enlarged and filled with oil and so also function as glands.

Comptonia asplenifolia, (L.) Aiton

The upper epidermis of the leaf of this plant consists of a layer of cells whose external walls are slightly arched outward and covered by a granular cuticle. The vertical walls of these cells are curvilinear. Over the veins the cells become rectilinear and elongated in the direction of the vein bundles. The mesophyll shows dorso-ventral differentiation into a broad palisade zone and a narrow spongy parenchyma region. The palisade zone consists of usually two layers of somewhat loosely arranged columnar shaped cells, the outer layer having its elements much broader than the inner one. The spongy parenchyma region is composed of irregular shaped loosely arranged cells surrounding intercellular-air-spaces. Rosettes of calcium oxalate are common in many cells of the mesophyll. The vascular systems of the mid-rib and smaller veins run through this region. That of the mid-rib is supported above and below by an arc of sclerenchyme. Those of the smaller veins are connected with the upper and lower epidermis by means of elongated hypodermal elements, some of which contain rosettes of calcium oxalate. The lower epidermis consists of a layer of cells with a granular cuticle. Their external walls are arched outward in a more prominent manner than those of the upper epidermis. The cells of this epidermis consequently are distinctly papillose in character. Their vertical walls are somewhat more curvilinear than those of the upper epidermis. Numerous stomata are scattered irregularly amongst the epidermal cells. These are surrounded by neighboring cells. Both upper and lower epidermis show four kinds of hairs. Of these, two are simple and two glandular. One type of simple hairs and by far the more common is composed of a single cell whose wall is sclerified throughout. At its base, as also shown by Chevalier, (7, p. 118) sclerenchyme threads extend in between surrounding cells in order to strengthen its insertion. The second type of simple hair is furchate and really consists of two hairs arising from contiguous basal cells whose adjoining margins have become soldered at the base. The first type of glandular hair is also seen in *M. Gale* and consists of 3-4 superimposed cells whose terminal cell or one of its median cells becomes enlarged and filled with oil. The second type consists of a stalk of 3-4 cells bearing on its summit a several celled spherical head which contains oil.

COMPARATIVE MORPHOLOGY OF THE INFLORESCENCES AND FLOWERS.

The characteristic inflorescence of the plants of the Myricaceae is a catkin. For *M. cerifera*, *M. Carolinensis* and *M. Macfarlanei*, the catkins are partly formed the year before flowering below the leaves on last season's growing branches. For *M. Gale* and *Comptonia asplenifolia* they are formed on special branchlets, which, after the development of the stamens on the male individuals and the fruits on the female individuals, cease to grow, and, upon the descent of the pollen tube to the egg, they soon die up to the node whereon the most inferior catkin is inserted and often up to 2 or 3 nodes lower. Chevalier (7, p. 143) separated *M. Gale* (*Gale palustris*, Chevalier) from the *Myrica* genus because of this character and placed it in the genus *Gale*. The writer feels that this character is insufficient to warrant the establishing of a new genus. The special branchlets after their death are separated from the living part of the stem by several thickenings of cells filled with gummy lignin. No disarticulation of the dead part takes place.

M. cerifera, *M. Carolinensis*, *M. Macfarlanei* and *M. Gale* have staminate catkins which are borne on different plants from those which bear the pistillate catkins. They are, therefore, dioecious. In regard to *Comptonia asplenifolia*, this is frequently the case; but it also frequently happens that both staminate and pistillate catkins are found on the same plant, even on the same branch, so that this species is both dioecious and monoecious. On monoecious plants the pistillate catkins appear below the staminate.

Structure of Catkins and Flowers of Myrica cerifera, L

The male catkins of *M. cerifera* are cylindric and unbranched and vary from 6 to 18 mm. in length, and from 2.5 to 4 mm. in breadth. The peduncle and rachis are yellowish-red and show a few thinly scattered hairs as well as a scattering of orange-red and golden-yellow glands. The staminate flowers are inserted in the axils of bracts which are arranged along the rachis in a 2/5 spiral. Each staminate flower consists of 4-6 stamens. The filaments of these are coalescent in their lower half, free above and bear extrorse anthers on their free ends. The bracts are deciduous, reddish oval structures, rounded at the base and sometimes attenuated. They are scarious, slightly thin hairy along the margin and glandular hairy beneath. The bracteoles are caducous, 1 to 2 in number, sometimes absent, deciduous, and inserted on the staminal column at different heights. The female catkins are short and oblong and attain a length of 5-10 mm. at the time of flowering. They develop more slowly than the male. Each pistillate flower consists of 2 carpels fused in their ovarian portion to form an ovate one-chambered compound ovary, which is narrowed above into two elongated spreading styles and stigmas. The pistillate flowers are arranged along a rachis in a 2/5 spiral. Each is inserted in the axil of a deciduous bract and accompanied by four deciduous bracteoles.

Myrica Carolinensis, Miller

The catkins of this plant possess a peduncle and rachis more hairy than those of *M. cerifera.* Furthermore, they have but one kind of glandular hair, the golden-yellow balloon type. The male catkins (Plate 84. Fig. 11) are 6-8 mm. long and about 5 mm. wide. The writer has found the staminate flowers of the male catkins with anthers in the tetrad state at Noroton, Connecticut, April 27, 1914. He has also observed them with mature pollen and ready to dehisce at Wildwood, N. J., May 13, 1914. In material collected at Clementon, N. J., April 23, 1915, the writer has observed tetrads and young pollen. Each staminate flower consists of 4-8 stamens whose filaments are welded together similarly to those of *M. cerifera* in half of their length, thus forming a staminal column which is inserted in the axil of a bract. The bract is thin hairy along the margin and yellow glandular hairy beneath. Bracteoles are either present or absent.

The female catkins (Plate 84. Fig. 10) are 8-10 mm. long and oblong in shape. Each pistillate flower inserted thereon consists of 2 carpels fused in their ovarian portion to form an ovate one chambered compound ovary, which is extended above into 2 spreading styles and stigmas. It is inserted in the axil of a greenish oval-lanceolate bract. The bract is obtuse at its summit, slightly hairy along its margin, and conceals two small deciduous bracteoles which are situated in its axil.

Myrica Macfarlanei, Youngken

The male and female catkins of the hybrid between *M. cerifera* and *M. Carolinensis* resemble those of *M. cerifera* in possessing both orange-red bowl-shaped and golden-yellow balloon-shaped glandular hairs on both catkin axis and the bracts. Other characters are still under investigation by the writer.

Myrica Gale, L

The male catkins of this plant are cylindrical (Plate 84. Fig. 9) 10-15 mm. long at the time of flowering. The staminate flowers each consist of 3-6 stamens with distinct filaments bearing adnate yellow anthers. The filaments are extremely short and are inserted in the axil of a reddish brown, broadly oval to sub cordate bract.

The female catkins (Plate 84. Fig. 8) are ovoid oblong, obtuse, from 10-20 mm. long and 6 mm. wide at the time of maturation. Each female flower consists of 2 carpels fused in their ovarian portions, each being terminated by a style and stigma. The ovary is flanked by two lateral bracteoles which develop into aeriferous floats upon its maturation.

Comptonia asplenifolia, (L.) Aiton

The male catkins are cylindrical and at the time of flowering attain a length of 20-25 mm. They are clustered at the ends of special branches in numbers ranging from 5-10 on each branch. Each staminate flower usually consists of four stamens (occasionally 5) whose filaments are distinct and short and bear reddish puberulent anthers. It is inserted in the axil of a bract which is not accompanied by bracteoles. The bracts along the catkin axis are oval to oval-lanceolate, generally 3 mm. long, terminated at the summit by a long point, brown and scarious, showing long white hairs along their margin and golden-yellow glands and thin simple hairs below.

The writer has observed the anthers in the pollen mother stage in material collected at Clementon, N. J., November 10, 1913, in the tetrad and mature pollen stage in material collected at the same place April 23, 1915.

The female catkins are ovoid and much smaller than the male, attaining a maximum length of 5 mm. at the time of flowering. They are grouped toward the end of special branches on pistillate plants or are frequently found below the staminate catkins on monoecious plants. Each pistillate flower consists of two carpels fused in their ovarian portion, and each terminated by a long red style and stigma. The ovary is fllanked laterally by two bracteoles which develop at their base and later along their margin small laciniate outgrowths.

COMPARATIVE MORPHOLOGY OF THE FRUITS

M. cerifera, L

Fruits small, spherical, bluish-white, from 2-3 mm. in diamter, covered almost to their summit by fleshy knob shaped glands which are devoid of a covering of wax in their young condition, but later are entirely covered over by that substance. Apex of mature fruits, aceriferous. Two-hundred fruits which the writer gathered at Palermo, N. J. November 7, 1914 weighed 2.86 gms. Fruits of one season past alone adhere to the axes of the catkins.

M. Carolinensis, Miller

Fruits larger than those of *M. cerifera*, spherical , tomentose hairy in their young condition, covered over early, as those of *M. cerifera* by a thick exudation of wax, traversed at maturity by a portion of the persistent hairs and having a diameter of 3.5-4.5mm. Apex of mature fruits, ceriferous. Two hundred fruits which the writer gathered at Palermo, N. J. November 7, 1914 weighed 9.27 Gms. The fruits of two seasons past adhere to the persistent catkin axes.

M. Macfarlanei, Youngken

Fruits intermediate in size, apex, weight and duration between those of *M. cerifera* and *M. Carolinensis*, having a diameter of 3-4 mm. Apex of mature fruits pitted. Two hundred fruits which the writer gathered at Palermo, N. J. November 7, 1914 weighed 6.7 Gms. The fruits of one season past and several of the previous season adhere to the persistent catkin axes.

Careful study has been made by the writer of stages in the development of the fruits of the above types. These will be described as one category seeing that they closely agree with each other.

In early June the maturing wall of each ovary has already developed a series of striking and complex glandular hairs of the nature of tubercular emergences. Each is a knob-like expansion of the fruit wall into which a copious prolongation of subjacent mesocarp tissue has spread but further, from an abundant and densely anastomosing complex of vascular bundles that ramify through the outer half of the maturing mesocarp, fine vascular diverticula composed of 2 or 3 spiral tracheae along with delicate elongated sieve-like elements pass through the stalk of the emergences and end in a slight swelling in its middle. The epidermis at this time is comparatively shallow and thin walled, while from the junction of the epidermis with the base of each emergence, elongated unicellular hairs spring, which form a basket like system, round and upon which copious wax exudations subsequently become aggregated.

At this time the mesophyll is divisible into two recognizable zones, viz.: an outer irregular and large celled region consisting of about 12-13 layers and an inner smaller and more round celled tissue of more numerous layers. Prolonged into the former from the point of attachment of the fruit with the axis is a vascular tissue that on entering the outer layer ramifies abundantly and as above stated gives off delicate diverticula to the different emergences. At this time only slight indication is observed of a difference in cell contents between the outer and the inner zone. The endocarp is a shallow and delicate layer that from now on becomes less and less conspicuous. By mid June or soon thereafter striking changes begin to appear. The epidermis, as well as the mesophyll cells of the emergences, the general epidermal (epicarp) cells of the fruit wall and the outer zone of the mesocarp have all enlarged steadily and become filled with an abundant secretion which assumes a bizarre coloration when stained with Safranin and Methyl Green. Tints varying from neutral-gray through pink, crimson, crimson-green, green-blue, yellow and brown are all present in distinct but neighboring cells and though the writer has as yet been unable to apply abundant tests, the above coloration suggests the formation and presence already of the palmitic, myristic and stearic acids already tested for and recorded by pharmacists and synopsized by Chevalier (7, p. 159).

At this time the inner layer of the mesocarp contracts conspicuously with the last, its cells remaining small, thin walled, rounded and its cavities filled with delicate protoplasm.

A further stage in the maturation of the fruits is noted by the middle or latter part of July. Each knob-shaped emergence has developed around itself an abundant waxy layer which, by hardening, gives a bluish white color to the fruit surface. The mesophyll cells of these hairs, as well as 3 to 6 of the innermost cell layers of the outer zone, become loaded with cell contents that assume a uniform red or reddish-green hue which even heightens the bizarre coloration noted above. By this time also the cells of the inner zone have become largely thickened from within-outward by lignified thickening and the cells themselves, having increased greatly in size and become markedly sinuous in outline, assume a bright red staining with Safranin.

The thickening process clearly proceeds in centrifugal fashion for in mid or late July the inner walls, surrounding the ovarian cavity, may be highly lignified and stained a bright red hue, while as yet the external cells adjacent to the outer zone are little, if at all, altered in shape or lignified. Progressive lignification of this area gives rise by mid August to the extremely hard scleroid fruit layers that efficiently protect the enclosed seed.

Meanwhile steady excretion of wax takes place over the cells of each emergence and these later have so grown together as to form a complete coating around the fruit wall proper. So between the abundant wax excretion and the close apposition of the wax secreting emergences, the entire surface of each emergence assumes a uniform blue gray color and is coated over by a rather brittle waxy investment that readily crumples to pieces when slightly pressed between the fingers.

This investment forms an admirable defensive covering alike against intense insulation, the attack of fungoid spores, and the destructive action of caterpillars and other animal enemies.

Gross Structure of the Fruits of *Comptonia asplenifolia*, Aiton

The fruit of *Comptonia asplenifolia* is an akene which is subtended by an involucral cupule consisting of 8 linear subulate bracteoles. The akeneal portion is ovoid-oblong in shape, obtuse at the apex, light-brown and shining. In association with other akenes and their bracteoles on the catkin axis, it forms a bur-like structure.

Gross Structure of the Fruit of *Myrica Gale*, L

The fruit of *M. Gale* is a keeled nut consisting of the ripened ovary accompanied by two accrescent bracteoles which have formed aeriferous floats.

Prevalence of Actinomyces in the Fruits

Actinomyces myricarum Youngken has been observed by the writer in its most luxuriant form in the cells of the middle fruit wall of the various species studied. Here it can be recognized best in thin hand sections stained with Safranin and Methyl Green in the form of rosettes almost filling the cell lumina. When the fruits fall to the ground and subsequently break open their walls, the organism probably makes its way from the infected cells into the soil where it spreads through wide areas infecting the roots and stems of other Myricas and producing characteristic lesions.

Taxonomy of the Myricaceae of the Eastern United States

Myricaceae (Lindley) *Bayberry Family*

Myricaceae, Lindley, Nat. Syst., ed. 2, 1836, p. 179;
Bentham and Hooker, Genera Plantarum, vol. 3, p. 400;
Cas. de Candolle, Prodromus, vol. 16, 2d p., p. 147.
Engler, Pflanzenfamilien, t 3, p. 26.

Myriceae, L. C. Richard, Anal. Fr., 1811, p. 493;
H. Baillon Hist. Plant., t. 6, p. 245.
Galeaceae, Bubani, Fl. Pyr., 2, 1899, p. 49.

Dioecious or sometimes monoecious, aromatic, resinous shrubs or trees with watery juice and possessing underground branches which arch downward then upward producing many suckers. Roots fibrous and bearing many short rootlets upon which are frequently found coralloid clusters of tubercles containing the *Actinomyces myricarum*, Youngken. Leaves alternate, revolute in vernation, serrate, irregularly dentate, lobed or entire, rarely pinnatifid, pinnately and reticulately veined, pellucid punctate, evergreen or deciduous, generally exstipulate, rarely stipulate. Flowers naked, unisexual, monoecious or dioecious, in the axils of unisexual or androgynous aments from scaly buds formed in the summer in the axils of the leaves of the year, remaining covered during the winter and opening in March or April before or with the unfolding of the leaves of the year. Staminate flowers in elongated catkins, each consisting of 2-8 stamens inserted on the torus like base of the oval to oval-lanceolate bracts of the catkin, usually subtended by 2 or 4 or rarely by numerous bracteoles; filaments short or elongated, filiform, free or connate at the base into a short stipe; anthers ovoid, erect, 2-celled, extrose, showing longitudinal dehiscence. Pistillate flowers in ovoid or ovoid-globular catkins. Gynoecium of two united carpels on a bract. Ovary sessile, unicellular, subtended by two lateral bracteoles which persist under the fruit or by 8 linear subulate bracteoles, accrescent and forming a laciniate involucre inclosing the fruit; styles short and dividing into 2 elongated style arms which bear stigmatic surfaces on their inner face; ovule orthotropous, solitary, with a basilar placenta and superior micropyle. Fruit an akene or ceriferous nut. Pericarp covered with glandular emergences which secrete wax or fleshy emergences, smooth and lustrous or smooth, glandular. Seed erect, exalbuminous, covered with a thin testa. Embryo straight, cotyledons thick, plano-convex; radicle short, superior.

There are two distinct genera of this family, eg. Myrica and Comptonia.

Characters of the Genera and Species

Genus: *Myrica*, L., Species Plantarum, 1024 (1753)

Mostly dioecious, evergreen, sub-evergreen or deciduous aromatic shrubs or trees with entire, dentate, or lobed glandular-dotted, exstipulate leaves; scales surrounding the ovary generally 2-4 very short. Stamens usually 2-6 in the axil of each bract. Ovary covered with ceriferous glands or fleshy non-ceriferous emergences with bracteoles adherent or non-adherent. Fruit an akene or ceriferous nut.

M. Gale, L.—Leaves membranous, fragrant, deciduous, petiolate, serrate near the summit; catkins borne on special deciduous branches; ovary flanked by 2 entire bracteoles which develop into aeriferous floats; wood consisting largely of tracheids.

M. cerifera, L.—Leaves coriaceous, fragrant, evergreen, petiolate, generally lanceolate-cuneate, covered on both surfaces with numerous orange-red and golden-yellow glands; fruits aceriferous at apex, 2-3 mm. in diameter.

M. Carolinensis, Miller—Leaves membranous, fragrant, deciduous, petiolate, elliptic-obovate, bearing numerous golden-yellow glands on both surfaces or glands absent on upper surface. Fruits entirely ceriferous at apex and 3.5 mm.—4.5 mm. in diameter.

M. Macfarlanei, x Youngken.—Leaves subcoriaceous, subevergreen, fragrant elliptic-obovate to lanceolate-cuneate, bearing a few orange-red and golden-yellow glands on their upper surface, or golden yellow glands only and numerous glands of both kinds on the lower surface. Fruits somewhat punctate at the apex and 3-4 mm. in diameter.

M. inodora, Bartram.—Leaves coriaceous, evergreen, petiolate generally oblong-obovate, inodorous. Rachis of the pistillate catkins glabrous. Fruits 5-7 mm. in diameter.

M. cerifera var pumila, Michaux.—Leaves coriaceous, evergreen, fragrant, obovate to linear-oblanceolate, sessile, or with a petiole 1-2 mm. long. Fruits subglobose, 3.5-4 mm. in diameter.

Genus: *Comptonia*, Banks, Gaertner. Fr. and Sam. 2:58 pl. 90 (1791).

Monoecious or dioecious aromatic shrubs with pinnatifid, stipulate, membranous leaves. Stamens commonly 4, occasionally 3-5, filaments free, short and not accompanied by bracteoles. Female flowers constituted by a bud carrying an ovary at its summit, flanked laterally by two bracteoles which develop at the base of their ventral surface and later on their margins small laciniate accrescent outgrowths. Fruit an elongated akene, surrounded until it falls from the catkin by a laciniate cupule formed by the development of two lateral bracteoles and the outgrowth which they have produced. Spikes of fruits globular, spiny and bur like. Edpidermis of fruit composed of stone cells of a shining black at maturity. Of this genus there is but one species, *Comptonia asplenifolia*, (L) Aiton.

For other and more detailed characters of the various species indigenous to the eastern United States see chapters on Comparative Morphology.

COMPARATIVE DISTRIBUTION

Myrica cerifera L.[1] grows in brackish marshes, in sandy soil on the border of brackish ponds, estuaries and near the sea from as far north as the Tuckahoe River, N. J., through Southern Jersey, Maryland, Virginia, as far south as Southern Florida, west through the Gulf States to the shores of Arkansas Bay in Texas and northward in the region west of the Mississippi tothe valley of he Washita River in Arkansas. Dr. Macfarlane and myself collected it along the banks of the Tuckahoe River, around the marshes and ponds bordering Petersburg road, at Palermo, Rio Grande, Wildwood, Cape May and Ocean City, N. J.

Myrica cerifera pumila (Michaux)[2] is found in low, sandy Pine Barren regions of South Carolina, Georgia and Florida, and on dry sand, and hills in Northern Alabama, Eastern Texas, Northern Louisaina and Southern Arkansas.

Myrica Carolinensis (Miller)[3] thrives in sandy, or sterile soil chiefly near the coast, but also in inland swamps and pine forests, from Labrador to Florida and Louisiana, on the border of the Great Lakes and in Indiana. We collected it at Noroton, Connecticut and at Tuckahoe, Clementon, Albion, Palermo, Ocean City, Rio Grande, Wildwood, Wildwood Junction, and Petersburg in N. J.

Myrica Macfarlanei (Youngken) (*M. cerifera* x *M. Carolinensis*) grows in the sandly soil of swamps, and on pine and holly forests near the sea in Southern New Jersey. We have found it at Palermo, Rio Grande, Wildwood, Tuckahoe, Cape May and Ocean City, wherever both of its parents—*M. cerifera* L. and *M. Carolinensis* Miller—abound.

Myrica Gale (L)[4] is widely distributed through northern regions, from Labrador and Newfoundland as far south as Warren County, New Jersey, from the Atlantic to the Pacific, and in eastern mountain regions to Virginia.

It was collected in 1913 on the border of back waters occasionally communicating with the sea at Peaks Island and Chebeague in Casco Bay, Maine, and in 1899 on Martha's Vineyard, Massachusetts by Dr. John M. Macfarlane.

Myrica inodora (W. Bartram)[5] is found in deep swamps in the vicinity of Mobile and Stockton, Alabama, near Poplarville, Mississippi in the valley of the Pearl River, and near Appalachicola, Florida.

Comptonia asplenifolia L. (Aiton)[6] thrives in dry, sterile soil and is widely distributed from Nova Scotia to Saskatchewan, and southward to North Carolina and Tennessee. We have found it forming a large part of the underbush associated with *Pteris aquilina* near Clementon, Albion, Palermo and Wildwood Junction, N. J., along Mainville Road, near Mainville, and at Strafford, Penna.

SUMMARY

1. Along the eastern seaboard of the United States there occur five good species of *Myricaceae* and a hybrid between two of these.

2. *Myrica cerifera* varies from a low shrub to a tree 12. m. high and extends northward, contrary to past statements, as far as Tuckahoe, New Jersey. The author finds this species to be evergreen and wholly confined to coastal regions within sight of the sea.

3. *Myrica Carolinensis*, with which the previous named species has often been confounded, is strictly deciduous except when strong basal shoots are formed. The leaves on these may be sub-evergreen.

4. The lanceolate leaves of *Myrica cerifera* drop without assuming a copper red color. The elliptic obovate leaves of *Myrica Carolinensis* assume a greenish-brown hue on an extensive scale in October and November previous to leaf fall. This species is of wide distribution along the coastal plain and even ascends to 1200 feet at Mount Desert.

5. Hybrids between the two above species (*Myrica Macfarlanei*, Youngken) are frequent where both parents abound. The leaf characters of this are averagely intermediate in duration, shape, thickness and coloration between the parents, *M. cerifera*, L. and *Myrica Carolinensis*, Miller.

6. *Comptonia asplenifolia* is distributed from Nova Scotia to Saskatchewan and southward to North Carolina and Tennessee. Its leaves are strictly deciduous.

7. *Myrica Gale* is distributed through northern regions, from Labrador and Newfoundland as far south as Warren County, New Jersey, from the Atlantic to the Pacific and in eastern mountain regions to Virginia. Its leaves are strictly deciduous

8. *Myrica inodora*, from the statements of authors, is evergreen. In height and aspect it resembles *M. cerifera*.

9. Seedlings are here for the first time described and figured from the cotyledonary stage onward for *M. cerifera*, *M. Carolinensis* and *M. Macfarlanei*. Their comparative morphology has been traced.

10. The author shows that from the seedling primary root of five to six months growth and from thence onward characteristic root tubercles are formed in the above named two species and their hybrid. The organism is found in all these to be an *Actinomyces* (here first described as *Actinomyces myricarum* of the author) that abundantly fills infested cells in the cortex of the tubercles, which owe their origin as arrested and modified roots to its irritant and invading action. As a result of cultures made from tubercles, the author concludes that good cultures of the organism can be secured on nutrient agar.

11. Since *Actinomyces* is frequently a virulent pathogenic organism in cattle and other domestic animals up to man, the author suggests the possible pathogenic relation of the *Myrica* organism to such animals. He, therefore, would regard the infesting organism as parasitic in relation.

12. In *Comptonia asplenifolia*, a similar Actinomyces organism is the primary infecting agent, but there often appears a mycelium producing fungus probably belonging to the Oömycetes.

13. As for the roots so for the stems of *M. cerifera*, *M. Carolinensis* and the hybrid, a careful comparative histological study has been made and details recorded as to the resemblances and differences of the hybrid and its parents. During this study oblique barred septa have been discovered separating the pitted vessels from each other.

14. Coccus-like forms that the writer believes to be involution forms of the infesting *Actinomyces* have been discovered in the cavities of the pitted vessels. Such seem to indicate the pathway of infestment taken by the *Actinomyces* in order ultimately to reach the fruit wall.

15. In the study of the leaves new structural details have been observed, but special interest attaches to the presence now recorded of orange-red bowl to saucer-shaped glandular hairs specifically characteristic of *M. cerifera* and in a reduced degree of *M. Macfarlanei* intermingled with golden-yellow glandular hairs of Chevalier. The latter only are present in *M. Carolinensis*. The hybrid, moreover, in general histology is shown to be more or less intermediate between the parents.

16. The last named conclusion regarding the hybrid and its parentage is further verified by comparative studies recorded for the stem and root.

17. Exact phytophenological records have been made as to the maturation of the floral parts and the period of blossoming in April and May.

Spore mother cell formation is completed by autumn of one year, but formation of tetrads proceeds in the different species studied from mid April to mid May in the Philadelphia neighborhood.

18. Successive stages in the formation of the fruits have been observed and studied histologically. A highly interesting detail here is the presence of the *Actinomyces* organism in beautiful radiate patches that fill many of the cells of the mid-fruit layer.

19. The structure, mode of origin and wax secretion of the knob-shaped glands covering the ovarian wall have been traced and the intermediate character of the fruits of the hybrid, as compared with those of the two parents above mentioned, has been demonstrated.

New and more exact diagnostic taxonomic descriptions than have hitherto been submitted by authors are presented above and in particular, the diagnostic characters of *M. cerifera*, *M. Carolinensis* and their hybrid have been fully elucidated.

SYNONYMS

For the convenience of reference, the writer presents the following table of technical and common names of the species considered together with their bibliography.

Myrica cerifera, L. (Species Plantarum, 1753, p. 1024).

Ligustrum americanum, lauri folio, Plumier, Descrip. pl. Americ. 1693; Tournefort, Institut., p. 597.

Myrtus brabanticae similis carolinensis baccifera, fructu racemoso semili monopyreno, Plukenet Almagestum, 1696, p. 250, t. 48, f. 9; Catesby, Carol 1, p. 69, t. 69.

Lychnochrodyophoros, Plukenet, Phytogr, tabl. 48, fig. 9.

Myrica cerifera, Miller, Gardener's Dict. Ed. 8; Sargent, Silva of N. Am., Vol. 9, p. 87 and plate 459; Britton and Brown's Illus. Flora of the U. S. and Canada, Ed. 2, vol. 1, p. 585.

Myrica cerifera arborescens, Castiglioni, Viag. negli Stati-Uniti 1790, vol. 2, p. 302; Michaux, Fl. Bor. Am., vol. 2, p. 228.

Lacistema Berterianum, Schult., Mant., 1822, vol. 1, p. 66.

Lacistema alterum, Spreng. Syst., vol. 1, 1825, p. 124.

Myrica heterophylla, Rafinesque, Alsograph. Am. 9 (1838).

Cerophora lanceolata, Rafinesque, Alsograph. Am. 11 (1838).

Myrica Carolinensis, A. Richard, Fl. Cub. vol. 3, p. 231 (1853).

Myrica microcarpa, Grisebach, Flor. Brit. W. Indies, p. 177 (1864).

Myrica altera, C. de Candolle, Prodr. 16, pt. 2, p. 595 (1868).

Morella cerifera, Small, Flora of S. E. U. S. ed. 2, p. 337 (1913).

Candleberry, Candel-wood, Candle Tree (Amer. sept., Plukenet l. c., p. 250).

Bayberry, Wax Myrtle, (Beringer, Notes on the genus *Myrica*, Am. Jour. Phar., May 1894).

Waxberry, Tallow-bayberry, Wax-myrtle, Candleberry, Tallow-shurb, Sweet Oak, Candleberry-Myrtle (Britton and Brown, Illus. Flora of U. S. and Canada, Ed. 2, vol. 1, p. 585 (1913).

Myrica Carolinensis, Miller (Dict. Ed. 8, 1768, n 3).

Myrtus brabanticae similis carolinensis humilior foliis latioribus magis serratis, Catesby, Hist. Carol., p. 13.

Gale americana humilis fructur coriandri, Burman, Herb.

Myrica pensylvanica, Loiseleur Deslongchamps in Nouv. Duhamel, vol. 2, 1802. p. 190. Chevalier Monograph Myricaceae, Mem. de la Soc. Nat. des. Sc. Nat. et. Math, de Cherbourg. vol. 32, p. 266.

Myrica cerifera B. media, Chapman, Fl. p. 427.

Myrica sessilifolia, Rafinesque, Alsog., 1836, p. 10.

Morella Carolinensis, Small, Fl. S. E. U. S., ed. 2, p. 337 (1913).

Small Waxberry, Bayberry, Britton and Brown Illus. Flor. of North. States and Canada. Ed. 2, vol. 1. p. 585.

Myrica cerifera var pumila, *Michaux* (Fl. Am. Bor., 1803, vol. 2, p. 228).

Willd. Sp. 4. p. 746.

M. sessifolia, Rafinesque, Alsograph. Am., 10 (1838).

M. pusilla, Rafinesque, l. c., 10.

Morella pumila, Small, F. S. E. U. S. ed. 2, p. 337 (1913).

Myrica inodora, *Bartram*, Trav., ed. 2 (1794), p. 403.

Myrica obovata, Chamisso ex H. Kew. in C. DC. l. c., p. 150.

Myrica laureola, Trecul ex H. Paris in C. DC., l.c ., p. 154.

Myrica Torreyana, Chapm ex H. Delessert.

Cerophora inodora, Rafinesque, Alsograph Am. 2 (1838).

Wax Myrtle, Sargent, Silva of N. A., Vol. 9, p. 91.

Myrica Gale, L. Spec. Plant, 1024 (1753).

Myrica palustris, Lamk., Fl. Franc. vol. 2, p. 236 (1778).

Myrica brabantica, J. E. Gray, Nat. arr. Brit. Pl. vol. 2, p. 249, (1821).

Gale belgica, Dumort., Fl. Belg., p. 12 (1827).

Cerophora angustifolia, Rafinesque., Alsog. Am. vol. 2, (1838).

Cerophora spicans, Rafinesque, l. c., 12, (1838).

Gale uliginosa, Spach. Hist. veg., vol. 2, p. 259, (1824) Bubani, Fl. Pyr., 1, p. 49.

Gale palustris, Chevalier, Monograph des Myricacees, Mem. d. e. Soc. Nat. des Sciences Nat. et Math. de Cherbourg. vol. 32, p. 177.

Gagel (of Old Germans).

Piemont, Piment royal (Centre de la France, Franchet).

Sweet Gale (Michaux).

Meadow Fern, Bog Myrtle, Dutch Myrtle, Willow Myrtle, Bay Bush (Beringer) Fern or Scotch-Gale, Sweet Willow, Golden osier, Moor-, bog-, Dutch- or Burton-myrtle (Britton and Brown, Illust. Flora of U. S. and Canada, vol. 1, p. 584).

Comptonia asplenifolia, Aiton, Hort. Kew. 334 (1789).

Myrica Comptonia, C. de Candolle, l.c., 151 (1864).

Myrica Gale Bentham, Pl. Hartweg., 336 (1857).

Liquidambar asplenifolia, Linnaeus, Syst. ed. 10, 1273 (1759).

Liquidambar peregrina, Linnaeus. Spec. 999 (1753).

Comptonia peregrina, Coulter, Mem. Torr. Club 5: 127, 1894, Chevalier Monog. des Myricacees, Mem. d. 1, Soc. Nat. des Sciences Nat. et Math. de Cherbourg., vol. 32, p. 193 (1902).

Myrica peregrina, O. Kze. Rev. Gen. Pl. (1891), p. 638.

Comptonia Ceterach, Mirb. in Duham. Arb ed. nov. 2, t 2.

Gale mariana, asplenii folio, J. Petiver, Mus. Petiv. 1695), p. 773.

Myrti brabanticae americana, foliorum laciniis asplenii modo divisis, Plukenet.

Almagest (1696) p. 250, t 100, f. 6, 7.

Sweet Fern, Sweet Ferry, Sweet Bush, Fern Gale, Spleenwort Bush, Beringer, Am. Jour. Pharm., May 1894, p. 222.

Meadow or Shrubby Fern, Canada Sweet Gale, Britton and Brown, Illust. Flora of North. U. States and Canada, ed. vol. 1, p. 586 (1913).

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5. W. Bartram: Travels, 2d ed., p. 403 (1794).
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14. Moeller: Beitrag zur Kenntnis der *Frankia subtilis* Brunchorst, Berichte der deutschen Bot. Gesellschaft, p. 215-224 (1890).
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Fig. 1 Seedlings of *Myrica cerifera*, showing root tubercles, collected at Wildwood, N. J., June 20, 1913.

Fig. 2 Seedlings of *Myrica Carolinensis*, also possessing root tubercles and collected on same date and at same place as above.

Fig. 3 Seedlings of *Myrica Macfarlanei* (*M. cerifera* x *M. Carolinensis*) gathered at Palermo, N. J., March 21, 1915. Note the position and frequency of the tubercles.

Plate LXXXII

Fig. 4 Entire plant of *Comptonia asplenifolia*, showing habit of growth. Collected near Clementon, N. J., July 15, 1913.

Fig. 5 Leafy branch of *Comptonia asplenifolia*, showing nature of stipulate leaves in mid-summer.

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Fig. 9 Staminate branch of the same plant, collected at the south end of Chebeague, in Casco Bay, Maine, by Dr. J. M. Macfarlane, August 1913.

Fig. 10 Branches of pistillate plant of *Myrica Carolinensis*, growing near the coast of Long Island at Noroton, Connecticut, April 27, 1913. Note total absence of leaves.

Fig. 11 Branches of staminate plant about 2½ feet high, growing near the sandy beach of Long Island shore, Noroton, Conn., April 27, 1913. Note total absence of leaves.

Plate LXXXV

Fig. 12 Staminate branch of *Myrica Macfarlanei*, collected at Wildwood, N. J., January 31, 1915. The leaves were nearly all discolored and somewhat shrivelled.

Fig. 13 Pistillate branch of *Myrica Macfarlanei*, collected as above. Note that many of the fruits are still adhering to the catkin axes on the second year's wood. Many of the fruit bearing axes of several seasons past are still adhering to the wood.

Plate LXXXVI

Fig. 14 Transverse section of the root of *Myrica Carolinensis* x 25.
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Fig. 16 " " " " " " *Myrica cerifera* x 13.5.
Fig. 17 " " " " " " *Myrica Gale* x 25.
Fig. 18 " " " " " " *Comptonia asplenifolia.*

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Fig. 21 " " " " " " *Myrica Carolinensis* x 13.5.
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Fig. 24 Transverse section through a tubercular root of *M. Carolinensis,* showing (a) the region of infestment of *Actinomyces Myricarum.*

Fig. 25 Longitudinal section through a tubercular root of *M. Carolinensis,* showing its nature of branching and the appearances of the hypertrophied cells containing *Actinomyces* rosettes.

Fig. 26 Section through an old tubercular root of *Comptonia asplenifolia,* showing the presence of a mycelium producing fungus having non-septate hyphae and probably belonging to the Oomycetes x 160.

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Fig. 30. Longitudinal section through a staminate catkin of *Comptonia asplenifolia.* X 10.

Fig. 31 Longitudinal section through a portion of the catkin of *Comptonia asplenifolia,* showing the anthers in the pollen mother stage.

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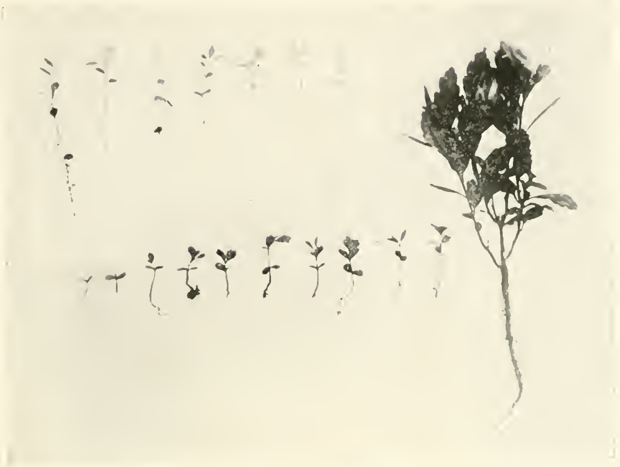
Fig. 33 Tubercular clusters on underground stem and roots of *M. Macfarlanei* observed by the author at North Wildwood, N. J., January 31, 1915.



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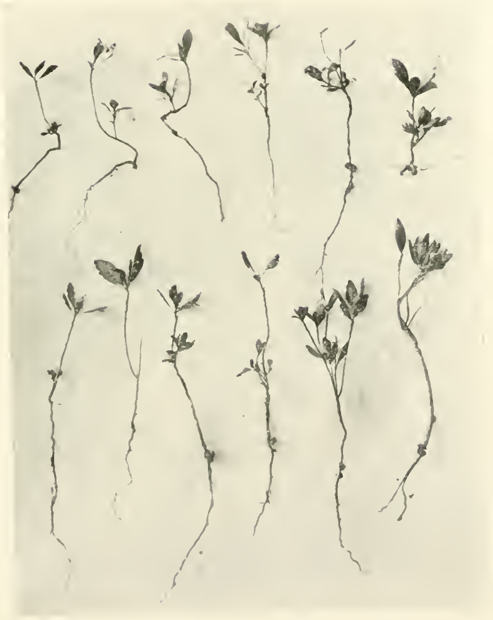
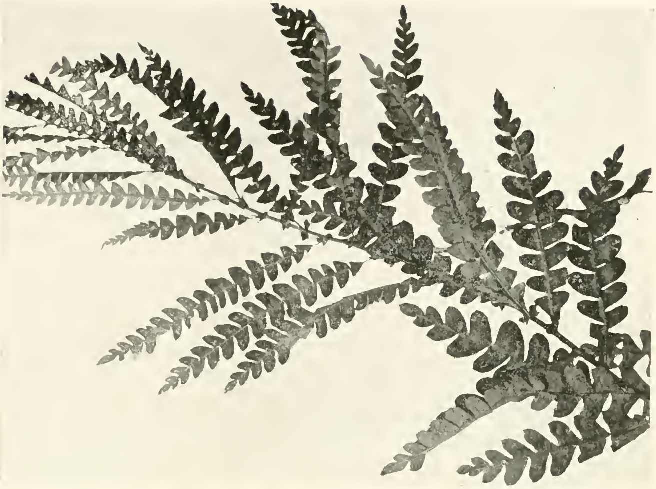


Fig. 3

YOUNGKEN ON MYRICACEAE

Fig. 5



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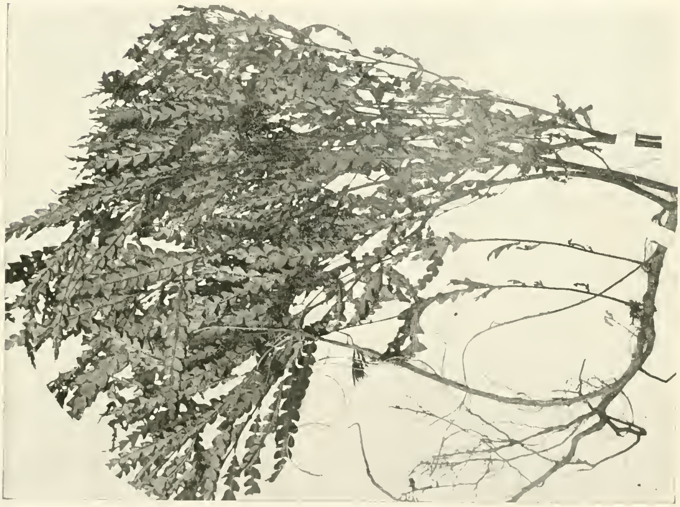

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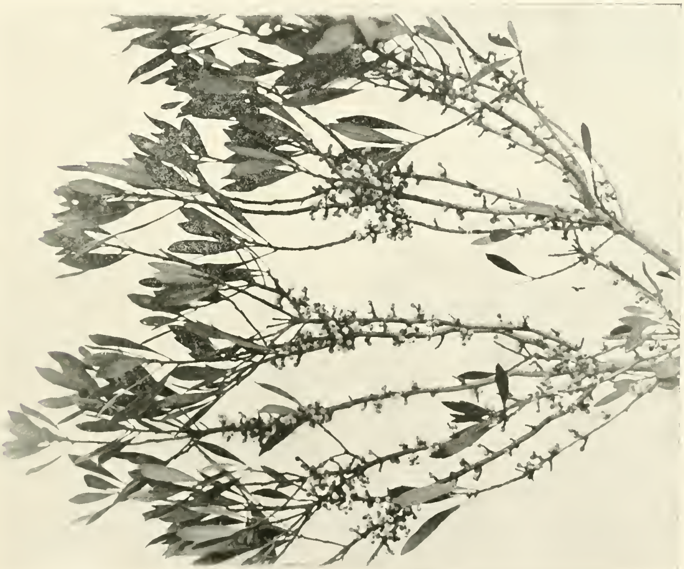

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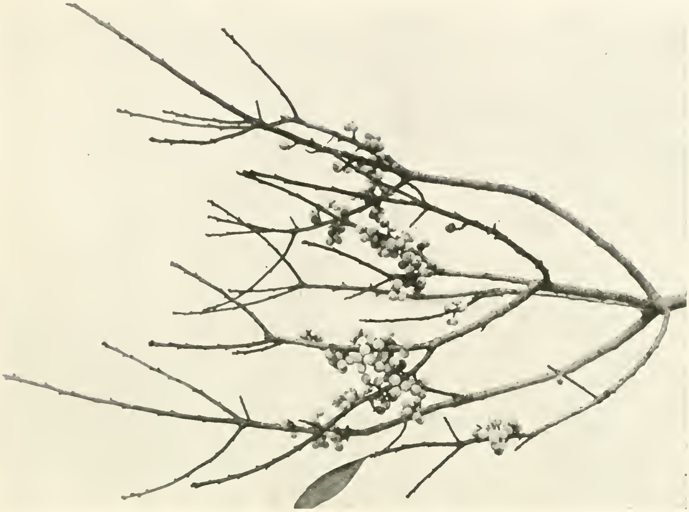

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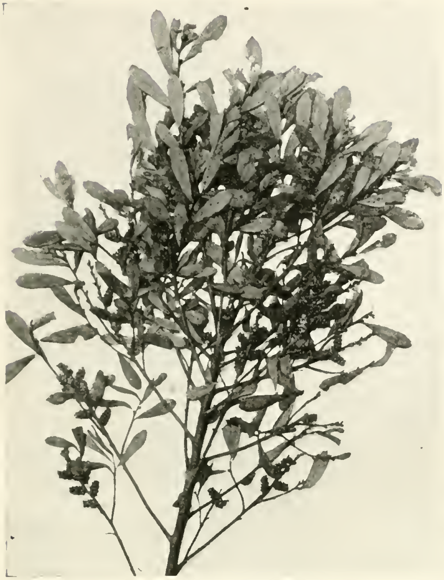
Fig. 8



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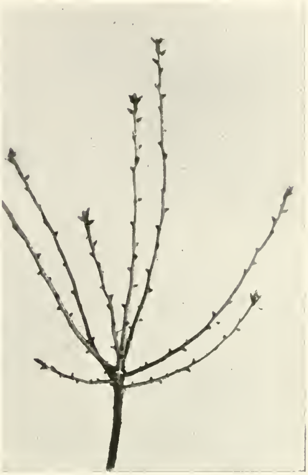

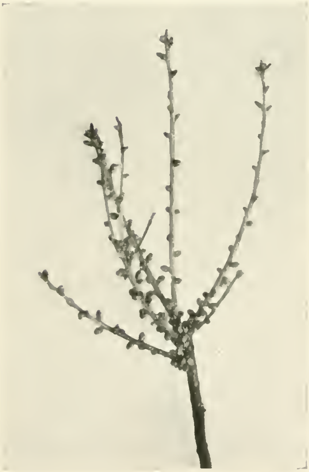

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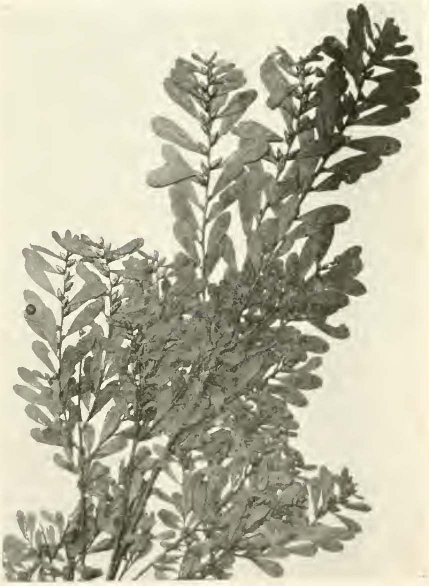

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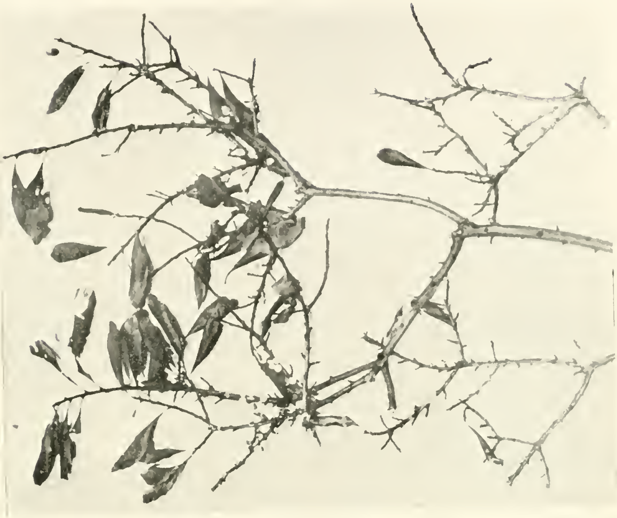

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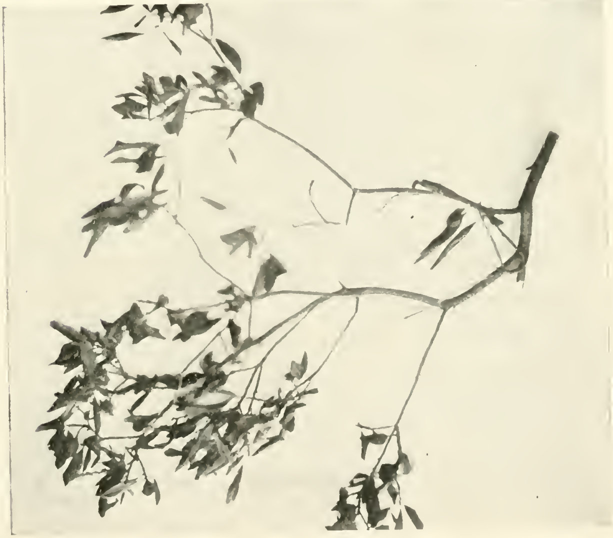

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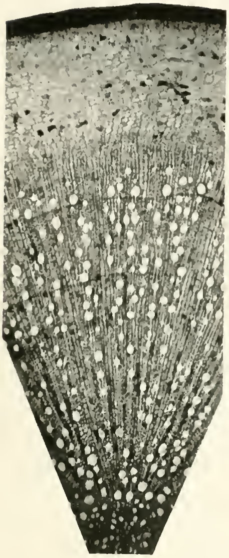

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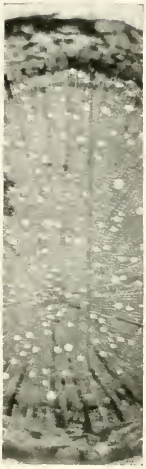

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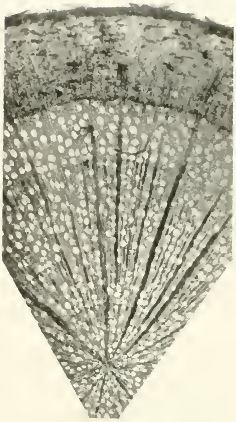

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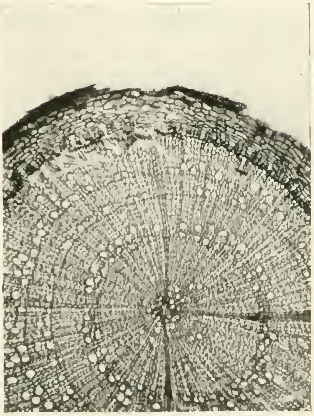

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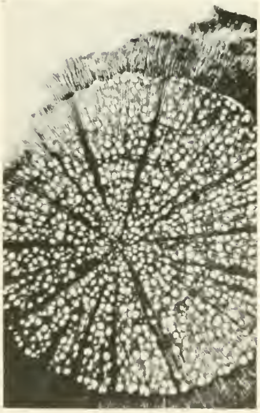

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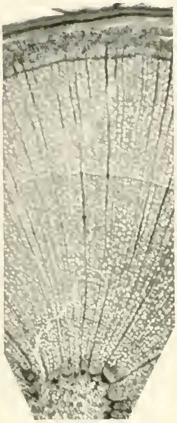

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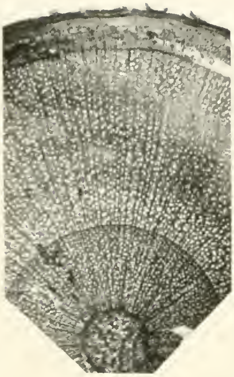

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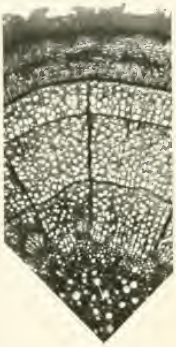

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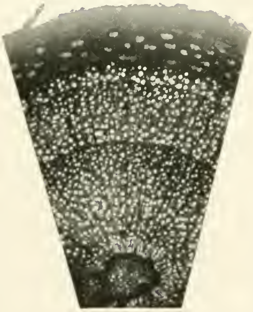

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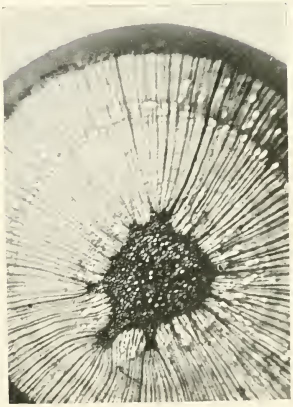

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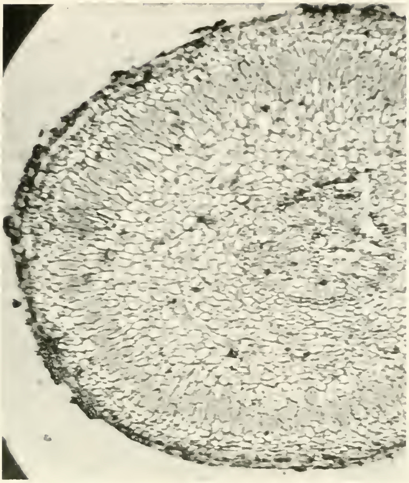

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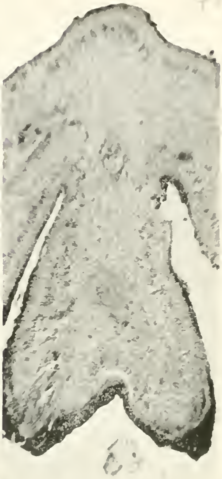

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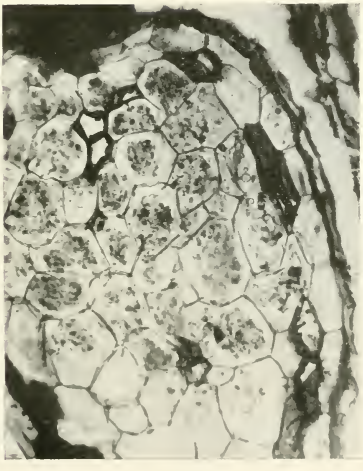

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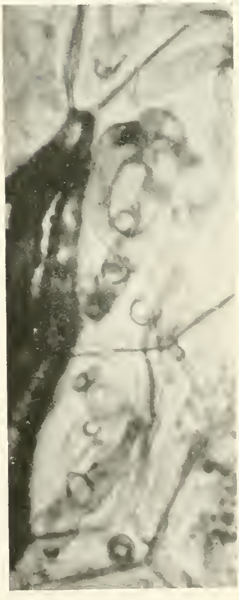

Fig. 27

YOUNGKEN ON MYRICACEAE

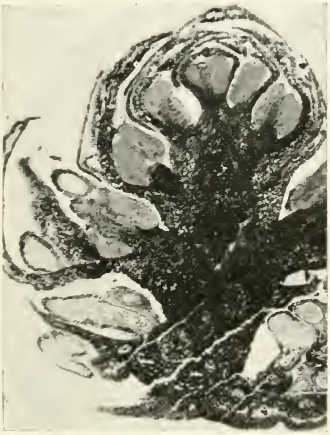

Fig. 28

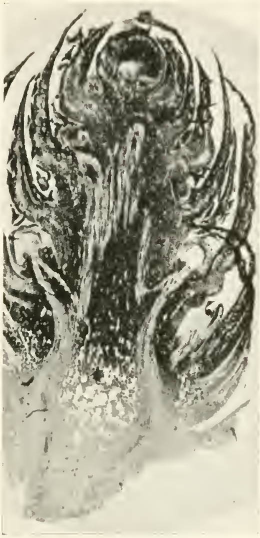

Fig. 29

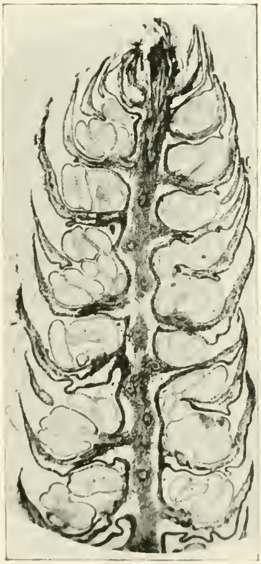

Fig. 30

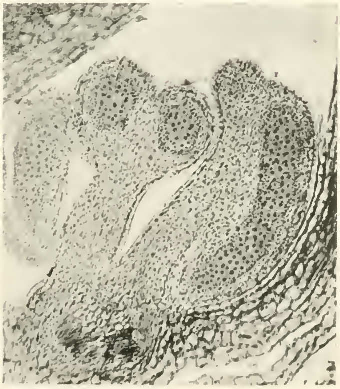

Fig. 31

YOUNGKEN ON MYRICACEAE

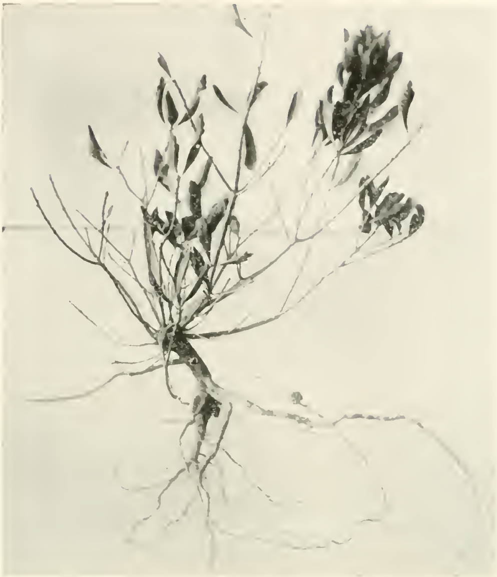

Fig. 32

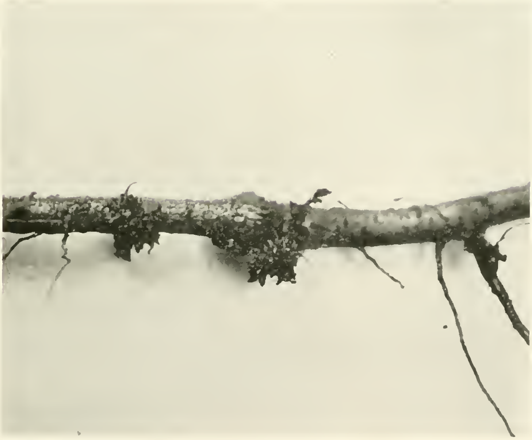

Fig. 33

YOUNGKEN ON MYRICACEAE

A COMPARATIVE STUDY OF *FLOERKEA PROSERPINACOIDES* AND ALLIES

BY

Alice M. Russell, B.S., M.S.

With Plates XCI, XCII

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INTRODUCTION AND HISTORICAL REVIEW

The purpose of this paper is to verify the affinities of *Floerkea proserpinacoides* in relation to other plant types.

Floerkea proserpinacoides was first described by Willdenow in 1801 in the "Neue Schriften der Gesellschaft der naturforschenden Freunde" (29). He did not give it a definite position in the natural orders of plants. In Barton's Compendium Florae Philadelphicae (30), it is placed in the division Hexandria Monogynia.

In 1833 Robert Brown described *Limnanthes Douglasii* before the Linnaean Society in June of that year. The abstract of the paper, quoted from the London, Edinburgh and Dublin Philosophical Magazine of General Science for July–December 1833, (31) is as follows:—"June 18, a paper was read, entitled, 'Characters and Descriptions of *Limnanthes*, a new genus of plants allied to *Floerkea*,' by Robert Brown, Esq. V.P.L.S. For specimens of the plant described, the writer is indebted to the Horticultural Society, and to Mr. David Douglas, F.L.S., by whom it was recently discovered in California.

"Mr. Brown was led more particularly to examine *Limnanthes*, from its resemblance to *Floerkea* of Willdenow, a genus which he had many years since investigated without being able to determine its place in the natural system. Examination proved these two plants to be so nearly akin, that they might perhaps be included in the same genus. They are here, however, separated and the two genera are considered as forming a family distinct from all those at present known.

"The place of the new family (Limnantheae) is not absolutely determined, but it is suggested that in two remarkable points of its structure, namely, the presence of glands subtending the alternate filaments and the existence of a gynobase it more nearly approaches to hypogynous families than to perigynous with which it has hitherto been associated.

"The following are the characters of the natural order, and of the two genera forming it:—

Limnantheae

Flos completus, regularis. Calyx 3-5 partitus, aestivatione valvata persistens. Petala 3-5, marcescentia. Stamina 6-10 insertione ambigua (hypo-perigyna) marcescentia. Filamenta distincta, 3-5 sepalis opposita basi extus glandula munita. Ovaria 2-5, sepalis opposita, cum stylo communi 2-5- fido mediante gynobasi connexa, monosperma, ovulo erecto, nucleo inverso. Achenia subcarnosa. Semen exalbuminosum. Embryo rectus; radicula infera. Herbae (Americae septentrionalis, paludosae) glaberrimae, alternifoliae exstipulatae, foliis divisis; pedunculis unifloris ebracteatis, apice dilatato basin turbinatum calycis simulante.

Limnanthes

Calyx 5 partitus. Petala 5, calyce longiora, aestivatione contorta. Stamina 10, ovaria 5. Herba (*Limnanthes Douglasii* Americae occidentali borealis.) foliis bipinnatifidis, pinnis sub-oppositis segmentis alternis.

Floerkea, Willdenow

Calyx 3 partitus. Petala 3 calyce breviora. Stamina 6. Ovaria 2 (raro 3) Herba (Americae orientali borealis). Foliis pinnatifidis segmentis indivisis."

Since the time when the above was written, it has been variously placed, some including it under the Geraniaceae as a sub-order *Limnantheae* (1). Others directly absorb it into the Geraniaceae (5). Recent authors accept Robert Brown's views, keeping it as a separate family (1). Some authors consider the two genera to be so closely allied as to compose one genus only; naming it from the first described genus of the family *Floerkea* (5).

SYNOPSIS OF FAMILY LIMNANTHACEAE

The family Limnanthaceae, as recently described by Axel Rydberg in North American Flora, Vol. 25, part 2, includes:—

I. *Limnanthes*

1. *Limnanthes alba* Hartw; Benth. Pl. Hartw. 301 (1848).
   Floerkea alba Greene, Fl. Fran. 100 (1891).
2. *Limnanthes floccosa* Howell, Fl. N. W. Am. 1:108 (1897).
3. *Limnanthes rosea* Hartw; Benth. Pl. Hartw. 302 (1848).
   L. pulchella Hartw. Jour. Hort. Soc. London 4:79 (1849) as a synonym.
   F. rosea Greene Fl. Fr. 100, (1891).
4. *Limnanthes Douglasii*, R. Br. Phil. Mag. III 2:40, (1833).
   F. Douglasii, Baillon, Adansonia 10, (1873).
5. *Limnanthes sulphurea*, Loud. Encyc. Pl. (1843).
   L. Douglasii Sweet, Br. Flower Gard. 2. (1838).
6. *Limnanthes versicolor* (Greene) Rydberg.
   F. versicolor, Greene, Erythrea 3:62. (1895).
7. *Limnanthes gracilis*, Howell, Fl. N. W. Am. 1, 108, (1897).
8. *Limnanthes pumila*, Howell, Fl. N. W. Am. 1, (1897).
9. *Limnanthes Macounii*, Trelease Mem. Boston Soc. Nat. Hist. 4:85, (1887).
   L. Douglasii Macoun Cat. Can. Pl. 3.502, (1886.)
   F. Macounii, Trelease in A. Gray Syn. Fl. 1, (1897).

II. *Floerkea*

1. *Floerkea occidentalis*, Rydb. Mem. N. Y. Bot. Gard. 1.268, (1900).
   F. proserpinacoides of S. Wat. Bot. King's Expl. 50, (1871).

2. *Floerkea proserpinacoides*, Willd. Neue Schr. Ges. Nat. Freunde. Berlin, 3.449, (1801).
   F. lacustris, Pers. 3 yn. pl. 1, (1805).
   Nectris pinnata, Pursch, Fl. Am. (1814).
   F. uliginosa, Muhl. Cat. 36, (1813).
   F. palustris, Nutt. Gen. 1, 229, (1818).
   Cabomba pinnata R. & S. Syst. Veg. 7, (1830).

Thos. Howell (2) has the above species of *Limnanthes* recorded excepting *L. sulphurea*. He does not recognise *F. occidentalis*. Asa Gray (5) accepts *F. proserpinacoides*. He places *Limnanthes* and *Floerkea* in the same genus, *Floerkea*. The species accepted by him are *F. Macounii*

F. Douglasii, *F. rosea*, and *F. alba*. *L. sulphurea* he considers synonymous with *L. Douglasii*. According to him *L. pumila* and *L. versicolor* are varietal forms of *L. Douglasii*.

The most stable species of the two genera composing this family (Limnanthaceae), accepted generally by all, are as follows:

LIMNANTHACEAE

1 *Floerkea*
    (a) *Floerkea proserpinacoides.*
        var. F. occidentalis.
2 *Limnanthes.*
    (a) *Limnanthes Douglasii.*
        var. L. sulphurea.
        var. L. pumila.
        var. L. versicolor.
    (b) *Limnanthes rosea.*
    (c) *Limanthes alba.*
    (d) *Limnanthes Macounii.*

DISTRIBUTION OF SPECIES

It will be seen from Robert Brown's key to the family that the two distinctions between the genera are that *Limnanthes* is a form with pentamerous symmetry, and is western in distribution, while *Floerkea* is a trimerous form, given as purely eastern in distribution. At that time *L. Douglasii* was the only species of *Limnanthes* known. There are, however, several other pentamerous forms, having a distribution identical with that of *L. Douglasii*, which have been described more recently. These pentamerous forms of *Limnanthes* have a purely western distribution—viz. California, Oregon, Washington (1).

There is a form of *Limnanthes* showing tetramerous symmetry described as *Limnanthes Macounii* (Trel. Mem. Boston Soc. Nat. Hist. 4, 85, 1887). Its distribution is restricted apparently to Vancouver Island, British Columbia (5) (1). But the discovery of a tetramerous form of *Limnanthes* is noteworthy, forming as it does a link connecting the pentamerous western forms with the trimerous *Floerkea*.

There is a species of *Floerkea* described by Rydberg as *Floerkea occidentalis*, resembling *Floerkea proserpinacoides* in all essentials, differing from it by a slight reduction in size of its parts as compared with *F. proserpinacoides*. It is probably a starved, or feeble form of *F. pro-*

serpinacoides of varietal rank. Some authors consider it as identical with *F. proserpinacoides* (12) (13).

Floerkea occidentalis is reported from Yellowstone Park, Utah, and Washington, ascending to an altitude of 2,000 to 2,500 m. (16); Wyoming, Colorado to California and Washington (1).

Those who consider *F. occidentalis* to be synonymous with *F. proserpinacoides* record *F. proserpinacoides* from:—

Washington, Ontario, south to California, Utah and Pennsylvania (12); Canada, Oregon, south in the east to Pennsylvania and Illinois, and in the west to California and Utah (5); Quebec to Ontario, Oregon, Pennsylvania, Tennessee, Missouri to Utah, and California (13); Oregon, California, Illinois, Canada and New England (2).

Those distinguishing *F. proserpinacoides* give its distribution as follows:—

In Washington, North Utah (18); Quebec to New Jersey, Tennessee, Missouri, Wisconsin (1).

From this it can be seen that *F. occidentalis* (*F. proserpinacoides*) overlaps the distribution regions of the pentamerous forms of *Limnanthes* and connects with the distributional area given for the tetramerous form of *Limnanthes* (*L. Macounii*). Thus, these two forms, if they are to be so considered, *F. occidentalis* and *F. proserpinacoides*, have a distribution ranging through California, Washington, Oregon, extending eastward through southern Canada to Ontario, Quebec, into New England and the middle Atlantic States to Pennsylvania. Here they reach, under the name of *Floerkea proserpinacoides*, the distribution accepted by the older authors for it in the East.

The whole group seems to have originated as a pentamerous one which still retains in *L. Douglasii* and *L. rosea* the attractive large flowers, which, to quote a rather popular Western Flora, make the western "meadows all a cream" in April. In its northern representatives a tendency toward reduction in the size of the flowers and the number of parts appears in the tetramerous species, *Limnanthes Macounii*. Overlapping the distribution of this tetramerous form is that of the trimerous one, *Floerkea occidentalis*, a condensed form which is by Piper and others regarded merely as a reduced type of *Floerka proserpinacoides*. This then stretches across the continent from California to Oregon thence to the Lakes, where a spur of distribution perhaps passed down tributaries of the Mississippi to Missouri, Illinois, Ohio and Tennessee. In making its way from Canada down through Quebec, Ontario, New England to Pennsylvania, the distributional lines may have been along the foothills of the Allegheny Mountains. The indications are that it is not found on the more recent areas which have become the coastal plains. Stone's Flora of New Jersey (17) records no locality for *Floerkea proserpinacoides*.

MORPHOLOGICAL AND ANATOMICAL STUDY OF FLOERKEA AND LIMNANTHES

Besides the close relation of the two genera, *Limnanthes* and *Floerkea*, as shown by their distribution, there is an even closer relation revealed by their structural similarities. This will be shown immediately in the discussion of the two forms *Limnanthes Douglasii* and *Floerkea proserpinacoides*, representative of the two genera.

Germination

Floerkea proserpinacoides shows hypogeal cotyledons and *Limnanthes Douglasii* shows epigeal cotyledons. The cotyledons are in both ovate and thick. Those of *Limnanthes Douglasii* are peculiar in that they possess at their tips a very noticeable water stomatic area as is the case in the leaflets. The cotyledons are green, functioning as leaves, and the presence of the water stomatic area on the leaf is not surprising since its habitat is usually an extremely moist locality (Fig. 1).

In *Floerkea*, a longitudinal section of the cotyledon shows at the tip, in the region corresponding to that of the water stomatic area in *Limnanthes Douglasii*, a distinct mass of epithem tissue (Fig. 2). This area can scarcely be considered to function as a water stomatic area in such colorless subterranean cotyledons. Rather it might be regarded as a vestigial structure. Its presence, however, is valuable as an indication of an ancestry of plants possessing epigeal cotyledons equipped with a water stomatic area at the tip as already traced in *Limnanthes Douglasii.*

This distinction between *Limnanthes* and *Floerkea* may possibly be broken down by the finding of a series ranging from epigeal through semi-hypogeal to hypogeal in the relation of their cotyledons. It may be that a reduced form, such as *Limnanthes Macounii*, might present this transition condition. No material for actual observation was obtainable and no references relating to the cotyledons were found.

Floerkea proserpinacoides this year (1917) germinated in the regions about Philadelphia in the week of March 21-28. By March 28, the plants were showing their first leaf in most localities. By the following week, the petiole of the first leaf had lengthened enormously. The second leaf was beginning to unfold and the flower buds were visible in the axils of the unfolding leaves. The first flowers appeared from April 9th on. The flowers are produced through April, one arising from the axil of each successive leaf. The present was an exceptional year for *Floerkea.*

From the end of April to the first week in May, it is usually dying out, and the seeds are ripening. This spring has, however, been extremely backward and now (May 25-29) the flowers are still appearing. The fruits formed from the earlier flowers are still green. The plants are beginning to show signs of decay, however. The leaves and stems are becoming withered and yellow. A few days of warm weather will probably end the unusually long period of growth of *Floerkea proserpinacoides* for this year.

The references made to the blooming season of *Limnanthes* indicate that the height of the blossoming period corresponds to that of *Floerkea.* April is the time for the appearance of the showy flowered *Limnanthes* in the western states.

Root

The root system of *Floerkea proserpinacoides* is characteristic of a shortlived reduced umbrophyte, that is, it consists of a few short fibrous unbranched roots. Besides the normal roots, adventitious roots may appear at the nodes on the stem, especially when the stem is procumbent upon the moist earth. These adventitious roots do not often appear above the third node. Plants growing upright in habitats more umbrophytic than hydrophytic do not show adventitious roots.

Limnanthes Douglasii shows a similar adventitious root formation at the first or second node.

In order to study microscopically the structure of the root in *Floerkea proserpinacoides* and *Limnanthes Douglasii*, material was imbedded in paraffin and sectioned. All sections were double stained with safranin and Delafield's haematoxylin unless otherwise stated.

A transverse section of a mature root of *Floerkea proserpinacoides* thus prepared (Fig. 3) showed: Externally a bounding layer of rounded epidermal cells, cuticularized externally. Within the epidermis is a homogeneous cortical region three to four layers in depth, composed of large round cells with thin walls. The innermost cortical layer is differentiated into a well-marked endodermis of elliptical cells thickened upon their inner and lateral faces. Within the endodermis is the pericambium, a single layer of large thin-walled cells This layer forms the outermost part of the vascular strand. The stele consists of a simple diarch bundle. The xylem plate is uniseriate, being formed of two to three spiral tracheae at each end, and three to four spirally thickened vessels centrally placed. The two phloem masses are of small thin-walled cells, not separable into the various phloem elements.

In the transverse section of the mature root (Fig. 4) of *L. Douglasii*, there appears: Externally an epidermis essentially as in *F. proserpinacoides*. The cortex region beneath is deeper, but is of cells of the same character. The endodermis and pericambium are as in *F. proserpinacoides*. The vascular strand is diarch, as before, but the xylem plate is not uniseriate The spiral tracheae are three in number, at the outer edges of the xylem plate, much as in *F. proserpinacoides*. The spiral vessels occupying the central part of the plate are arranged radially about a central cell. There are about ten of these vessels in *L. Douglasii*.

In a longitudinal section of the root tip region in *Floerkea proserpinacoides* the plerome cylinder is about 4 to 5 cells in width. Overlying the plerome is a regular periblem layer, which by its division gives rise to the plerome and periblem. Over the periblem of the apex region of the root is a crescentic group of cells which gives rise to both dermatogen and root cap, and can therefore be called a calyptrodermatogen layer. The root cap arising from the division of this layer is conical, and about five layers of cells thick in its deepest portion.

The longitudinal section of the root tip in *L. Douglasii* shows the same structure, differing only in having a wider plerome cylinder than *Floerkea*.

Stem

The stem of *Floerkea* is weak and flaccid. It remains green, and rarely branches in *Floerkea*. In *Limnanthes Douglasii* the stem is stouter and is much branched, especially at its base.

Sections of the stems of *Floerkea proserpinacoides* imbedded in paraffin were cut, and stained in safranin and Delafield's haematoxylin. In such transverse sections, there is an external epidermal layer of small rectangular cells, slightly cuticularized (Fig. 5). Beneath the epidermis is a layer of larger oblong cells, regular in shape, not thickened. Within this is the cortical region, 6 to 7 cells in depth. The cortical cells are large, round cells with thin walls. Throughout the cortical region are definite circular inter-cellular spaces surrounded by a sheath of cortical cells. The innermost cortical layer forms an indistinct endodermis. Within the endodermis is the vascular strand. This consists of a number of collateral bundles, separated from each other by pith or parenchymatous tissue. There is no ring of wood formed. The bundles consist of two or three spiral tracheae, and a varying number of spiral, or annular tracheids. The phloem is poorly developed, consisting of a few sieve tubes and fewer phloem cells, forming a slightly darker stained patch external to the xylem in the bundles. The two outermost layers of phloem cells form a loose, open-celled outer bast fiber region. *L. Douglasii* shows this more strongly developed. Between the phloem and the xylem, there is an easily recognizable large celled intrafascicular cambium. There is no interfascicular cambium shown however. The intrafascicular cambium shows division to form secondary wood. In most of the stems sectioned, 8 to 10 bundles were distinguishable. Several join, usually two to four at the ends of the ellipse of the bundle strand The bundles thus placed are those which pass off at the node into the petioles of the leaves. The other bundles remain distinct.

In the transverse sections of *L. Douglasii* stems which were cut free hand from herbarium material, and stained in safranin and methyl green, the structure was found to resemble *F. proserpinacoides* closely. The epidermis is as in *F. proserpinacoides*, excepting for the development of a thick cuticle. The same regular row of cells is present immediately beneath the epidermis. The cortex is of from 7 to 8 layers of large cells; intercellular spaces are rare, no air lacunae are present. The endodermis is indistinct. The bundles are more strongly developed, that is, the xylem elements are more numerous, and the phloem patches are larger. A complete ring of bundles is not formed, and little secondary wood is laid down. The two outer layers of the phloem form a poorly developed bast fiber region. The cambium is peculiar, its large thin-walled cells resembling markedly the cambium in *F. proserpinacoides*. The young stem of *L. Douglasii*, above the cotyledons, shows a number of air lacunae in the cortex corresponding to those in *F. proserpinacoides*. These do not appear in the older stems of *L. Douglasii* examined.

The bundles vary in number, in the stem of *F. proserpinacoides*, according to the level at which the sections were made. In the younger parts of the stem, the bundles may be four with four wide medullary rays between the bundles. This is also true in *L. Douglasii*. These bundles separate in the older stem into a number of bundles. At the nodes four bundles give off traces into the petiole.

Leaf

The leaves in *F. proserpinacoides* are alternate, exstipulate, and pinnately compound, with 3 to 5 leaflets. In *Limnanthes*, the leaves may be pinnate with 7 leaflets, to bipinnate as in *L. Douglasii*.

The first foliage leaf is trifoliate. The second leaf may be perfectly 5 foliate, but is more usually imperfectly divided, showing one terminal entire leaflet, and two lateral half-divided leaflets, or four entire leaflets. The third leaf is usually perfectly 5-foliate in *Floerkea*.

Leaflets were imbedded in paraffin and sectioned. These showed a characteristic umbrophytic structure. In transverse section there appeared:—An upper epidermis of rounded cells, not thickened (Fig. 6), containing stomata. Below the epidermis is a layer of loose palisade tissue, rich in chlorophyll, with many open intercellular spaces lying below the stomata. Below the palisade tissue is a narrow zone of mesophyll tissue containing the bundles. There are, in transverse section of a leaflet, a stronger midrib, two lateral strong veins, and two intermediate smaller veins. The vascular bundles are poorly developed, showing merely a few xylem and phloem cells. The lower epidermis is identical with the upper, excepting for the presence of more stomata. The veining in a leaflet consists of a midrib, branching at the base into two lateral branches running along the margin of the leaflet, and uniting with the midrib at the tip. From the margin lesser veins run toward the middle of the leaflet. A longitudinal section (Fig. 7) through the median part of a leaflet shows at the tip a single hydathode. From the end of the united bundles at the tip a mass of parenchymatous tissue spreads out in a fan-shaped mass. These cells are filled with a denser protoplasm than the surrounding cells. This is the epithem tissue of the hydathode. It lies immediately below a circular area of epidermal cells at the tip of the leaflet. The cells of this area differ from the ordinary epidermal cells in that they are oblong in shape and have smooth, straight walls. Stomata are more numerous than in the epidermis outside this region and are of smaller size (Fig. 8 and 9). The guard cells of these stomata are always open. The stomata themselves are more nearly spherical in outline than the normal stomata, which are elliptical in shape, and usually have their guard cells closed.

The water stomatic area at the tip in the older leaflets undergoes a breaking down in the epidermal cells, forming in this way a water pore (Fig. 7).

In both *Floerkea* and *Limnanthes* the leaves are glabrous. In *Limnanthes*, however, there are present tannin containing cells on the under surface of the leaf. They are long slender cells, running parallel to the axis of the leaflet. Sometimes they are present on the outer face of the sepals (Fig. 13). These are mentioned by Solereder (25). They give a positive reaction for tannin upon the application of ferric chloride. There are no tannin cells present in *Floerkea proserpinacoides.*

Flower

The flowers of *F. proserpinacoides* are solitary axillary. All the leaves, except the first foliage leaf, bear a flower in the axil.

In *F. proserpinacoides* the flowers are small and inconspicuous. They are trimerous in symmetry. There are three ovate acuminate green persistent sepals, united at their bases. Stomata are present in great numbers on both the outer and inner faces of the sepals. Water stomata are present upon the tip.

The petals are oblong and white, three in number, and only one-third as long as the sepals. Upon the base of the petals are a few blunt short unicellular hairs (Fig. 10).

The stamens are six, in two rows. The outermost row, opposite the sepals, have upon their outer side a gland at the base of the filament. The inner row, opposite the petals, are without glands. The stamens equal each other in height, and are shorter than the petals. The anthers are globose, and introrse, versatile, dehiscing longitudinally. The pollen is smooth and oval. The stamens with the petals are set on the inner edge of a flat disk of cells upon the receptacle.

The pistil consists of three separate carpels with a gynobasic style set between the carpels. There are usually only two carpels well developed, the third being a mere rudiment which is early crowded out by the two functioning carpels. The style rarely divides into three, mostly into two short style arms, each bearing a capitate stigmatic surface.

Sections of each young carpel show one anatropous ovule. The funiculus arises from the base of the receptacular tissue, runs up freely within the carpel and bears the inverted ovule (Fig. 11). When a third carpel is present, the ovule present is rudimentary, and imperfect. One carpel alone usually matures; maturation of two carpels is, however, not uncommon.

In sectioning the flowers at different stages, there are shown in the carpels and fruit certain peculiarities of structure. Over the carpellary wall there are papillae visible even to the naked eye. In transverse sections prepared from paraffin material these papillae are seen to be formed by pushing out of the large celled epidermis of the carpellary wall. These in the fruit cause the appearance described as "papillose," "rugose." These epidermal cells are peculiarly thickened on their outer walls by wart-like protuberances. Upon application of chlorophyll extract, these color green, but give no color reaction when phloroglucin is applied, showing that they are thickenings of cutin, not lignin. Within this epidermal layer in the carpel are four layers of large cells with thin walls, which form the remainder of the carpellary wall. The innermost layer of the carpellary wall is flattened, forming a distinct line of demarcation between the carpellary walls and a broad zone of large thin-walled cells. This is shown, by its reaction to iodine, to be a starch storing layer. In its outer part, lying nearer to the carpellary wall, are numerous small vascular bundles, cut transversely. This zone is, from all evidence at hand, a wide development of the tissue of the integuments surrounding the single ovule. The wide development and apparent fusion of the integuments, and the apparent single layer of the nucellar tissue, breaking down early in the development of the ovule, are characteristics resembling the development of the ovules of *Impatiens fulva*, as described by Mr. Franklin Carroll in the present publication ("The Development of the Chasmogamous and the Cleistogamous Flowers of *Impatiens fulva*"). *Floerkea* represents perhaps a more reduced state than that found in *Impatiens fulva.* Further careful study of the development of the ovule of *Floerkea* would be necessary, however, before a definite opinion on the matter could be expressed.

Within the fused integuments in the more nearly matured seed is a broken down granular substance, immediately surrounding the remnants of the embryo sac membrane. This represents the broken down nucellar tissue. Within the embryo sac is the embryo showing the cotyledons cut transversely, and the radicle between. In the spaces about the maturing embryo are shreds of the broken down endosperm tissue almost entirely consumed by the embryo at this stage of growth.

In *L. Douglasii* the flowers are solitary axillary. The flowers are 5-merous in symmetry, having 5 sepals, valvate in aestivation, green and persistent, united at the base as in the *F. proserpinacoides.* The petals are spatulate and about twice the length of the sepals. They are whitish or yellowish in color. On the base of the petal are unicellular hairs of the same type as in *Floerkea*, but longer. Fig. 10 gives the relative size of the hairs in *Floerkea* and *Limnanthes.* On the middle regions of the petals in *Limnanthes*, however, are hairs not present on the petals of *F. proserpinacoides.* These are longer than the petals of *F. proserpinacoides* and are shown in relative size in Fig. 10. These hairs are peculiar in their shape and walls. They are unicellular, and tapering. Their walls are covered with wart-like thickenings resembling the thickenings of the walls of the epidermis in the carpels of *Floerkea* and *Limnanthes.*

The stamens are 10, in two rows. The outer row, opposite the sepals, and not the row opposite to the petals, as is stated in some descriptions, are the ones which bear the gland, as in *Floerkea.* The stamens are shorter than the petals or sepals, and are all of the same length as in *Floerkea*.

The carpels are five in number, all well developed, and having between them the gynobasic style bearing five short style arms with capitate stigmas as in *Floerkea*.

The carpellary wall in *Limnanthes* has papillae as in *Floerkea*. Upon examination these show upon their outer epidermal cells minute thickenings of cutin as in *Floerkea* (Fig. 11). The much developed integument is present in *Limnanthes* also as in *Floerkea*, storing starch until late in the growth of the seed.

In going over a great number of plants of *Floerkea proserpinacoides* many were found to have on them flowers departing from trimerous symmetry. Most of these had four sepals, three petals, six stamens, and two carpels. The sepals upon examination showed by their venation that the four were derived by a splitting of one of the three usually present. Several were found with four sepals, four petals, six stamens, two carpels. One only was found with five sepals. It, however, had only three petals.

Conclusions

In the separation of the two genera forming this family the distinguishing features have been:—

Symmetry of flowers
Length of petals
Aestivation of the petals

In flower symmetry, however, there is now a gradation series. This begins with those having pentamerous symmetry, such as *L. Douglasii* and *L. alba*. The tetramerous flower of *L. Macounii* forms the transition type to the trimerous form described as *Floerkea*. In *Floerkea* constant variations from the tetramerous to the trimerous type have been found.

These range from imperfect tetramery in the sepals, to forms with the four sepals and four petals. One flower found approached pentamerous symmetry in possession of five sepals. The variations in these cases did not extend to the stamens and carpels. The stamens remained six in number, the carpels, as usual two to three.

In the length and aestivation of the petals there is also an intergrading series represented in the two genera:—

Limnanthes rosea seems to be the climax type, possessing as it does rose colored petals, much exceeding the sepals in length (sepals 7-8 mm. long; petals 12-18 mm. long). Petals convolute in aestivation.

Limnanthes Douglasii:—the petals are a more primitive color, white or whitish yellow, still much exceeding the sepals, yet not so large as in *Limnanthes rosea* (sepals 5-6 mm., petals 10-15 mm.). *Limnanthes versicolor* is about as *L. Douglasii* and is probably a hairy form of *L. Douglasii*. *L. alba* also has petals exceeding the sepals (sepals 6-8 mm., petals 10-15 mm.).

Limnanthes pumila forms a transition type from those whose petals exceed the sepals, to those with sepals exceeding the petals. It is given as having petals "scarcely exceeding the sepals." The petals too in this more nearly approach the oblong type found in forms with smaller petals.

Limnanthes floccosa comes next with "oblong cuneate petals, not exceeding the sepals."

In the tetramerous *Limnanthes Macounii* the petals equal the sepals. This forms a valuable transition species, being reduced both in the number and size of the parts (petals 3-4 mm., sepals 3-4 mm.).

In *Floerkea proserpinacoides* the petals are oblong and about the length of the sepals (sepals 3 mm.; petals 1-5 mm.), flowers trimerous, valvate in aestivation.

In *Floerkea occidentalis* the petals and sepals are reduced in size (petals 1 mm., sepals 2-3 mm. long). The petals are valvate in aestivation. This distinction is not important since it would be impossible from the size of the petals in *Floerkea* to have a convolute aestivation. The petals are so small and reduced that they do not overlap.

The two species of *Floerkea* are distinguished by Rydberg (1) on account of the different lengths of peduncles, thus:—

Peduncles longer than the petioles—*F. occidentalis*.

Peduncles rarely equalling the petioles—*F. proserpinacoides*.

This is not a difference to be depended on, for *F. proserpinacoides* does show the peduncles longer than the petioles. The plants exhibiting this were collected in different localities around Philadelphia. Rydberg and others who accept *F. occidentalis* do not report it from the region about Philadelphia.

From the anatomy of these two forms just described, *F. proserpinacoides* and *L. Douglasii*, it will be seen that they show a remarkable similarity in structure. Their correspondence in such minute details as the thickenings upon the epidermal cells of the carpellary walls, in the possession by both of the fused integuments about the ovule, which store food in the form of starch, of the subsequent absorption of the starch in the growth of the embryo, and the flattening of the empty cells by the enlarged embryo in the mature seed; by the presence upon the tip of the cotyledon in both of a water stomatic area, is striking. Similarity of structure in all of these parts indicates surely an extremely close relation between these two plants placed in separate genera. In comparing the anatomical features, it seems reasonable to look upon *Floerkea* as a type of plant evolved from *Limnanthes* by reduction. Thus: the stem shows a similar but reduced structure to that of *Limnanthes*; the glands upon the stamens in *Floerkea* are not so large as are those of *Limnanthes*, nor are the cells so rich in protoplasm. The hairs upon the base of the petals in *Floerkea* are shorter than those in *Limnanthes*, where they probably act as protecting hairs about the nectaries or glands at the base of the stamens between the petals. In *Floerkea* these probably do not function as in *Limnanthes*, since the petals are much reduced in size, and the hairs are too short to reach out and protect the gland at the base of the stamens. In the number and size of flower parts *Floerkea* shows reduction from *Limnanthes* as has been already traced. The geographical distribution already noted also suggests a continuous relation and close affinity between the pentamerous, tetramerous and trimerous forms in the two genera.

Until a complete series is made out, however, between the pentamerous forms with epigeal cotyledons and the trimerous forms with hypogeal cotyledons the two genera will have to remain distinct as Robert Brown viewed them.

The writer takes this opportunity to acknowledge her indebtedness to Dr. John M. Macfarlane for his advice and assistance in preparing this paper.

Summary

Two plants were studied microscopically and macroscopically as representing each one of the two genera of the family *Limnanthaceae*—*Floerkea proserpinacoides* and *Limnanthes Douglasii.*

In the anatomical features of the root, stem, leaf, flower and fruit, these two forms show a striking similarity of structure, *Floerkea proserpinacoides* indicating by its reduced members that it is a form derived from *Limnanthes* by reduction. In its present distribution, the family *Limnanthes* is represented in the west by species of *Limnanthes,* and in the east and west by *Floerkea.* Transition types are represented by various species of *Limnanthes* ranging from large attractive pentamerous types through a lesser tetramerous species to the trimerous flower types characteristic of the genus *Floerkea.* Since the genus *Floerkea* overlaps the distribution areas of the pentamerous and tetramerous forms it is likely to be a type evolved from them which worked its way through

southern Canada to northern New England and the Middle Atlantic States. Although the two genera are similar enough to form a single genus, until forms showing transition from the pentamerous type of *Limnanthes* with epigeal cotyledons, to those of the genus *Floerkea* with trimerous symmetry and hypogeal cotyledons, the two genera should remain perhaps as in Robert Brown's Classification.

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DESCRIPTION OF PLATES

Plate XCI

Fig. 1. Longitudinal section tip of cotyledon *Limnanthes Douglasii* (×220)

H = Epithem tissue below water stomatic area.
T = Bundle supplying epithem tissue.
S = Storage tissue of cotyledon.

Fig. 2. Longitudinal section tip of cotyledon of *Floerkea proserpinacoides* (×150).

H = Epithem tissue.
T = Vascular strand.
S = Storage tissue of cotyledon.
K = Remnants of seed coats.

Fig. 3. Transverse section root of *Floerkea proserpinacoides* (×220).

E = Epidermis.
C = Cortex.
D = Endodermis.
X = Xylem.
P = Phloem.

Fig. 4. Transverse section root of *Limnanthes Douglasii* (×220) labeling as above.

Fig. 5. Transverse section stem of *Floerkea proserpinacoides* (×80).

E = Epidermis.
C = Cortex.
L = Lacunae.
D = Endodermis.
Pi = Pith.
X = Xylem.
P = Phloem.

Plate XCII

Fig. 6. Transverse section leaflet of *Floerkea proserpinacoides* (×153).

T = Palisade tissue.
I = Intercellular space.
M = Midrib.

Fig. 7. Longitudinal section leaflet of *Floerkea proserpinacoides* (×153).

T = Palisade tissue.
H = Epithem tissue.
P = Mesophyll.

Fig. 8. Stomata from water stomatic area *Floerkea proserpinacoides* (× 200).

Fig. 9. Stomata from lower epidermis *Floerkea proserpinacoides* (× 200).

Fig. 10. A. Petal of flower of *Floerkea proserpinacoides*, showing hairs at its base (× 15).

B. Hairs from base of petal of *Limnanthes Douglasii* (× 15).

Fig. 11. Longitudinal section thru ovule of *Floerkea proserpinacoides* (× 150).

C = Carpellary wall.
N = Nucellus.
S = Membrane of embryo sac.
E = Egg.
F = Fused indusia.

Fig. 12. Carpels and gynobasic style of *Floerkea proserpinacoides* (× 25).

Fig. 13. Sepal of flower of *Limnanthes Douglasii*, showing cells with tannin contents (× 25).

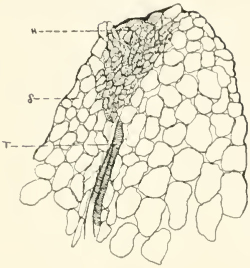


Fig. 1

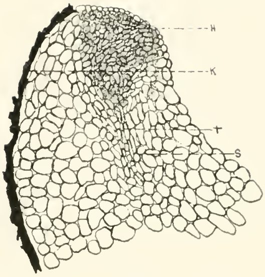


Fig. 2

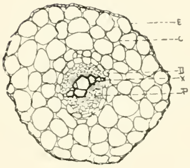
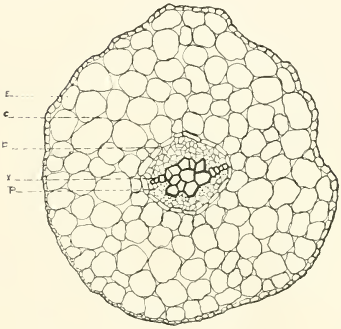


Fig. 3



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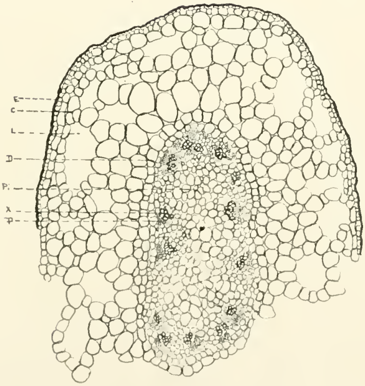


Fig. 5

RUSSELL ON FLOERKEA

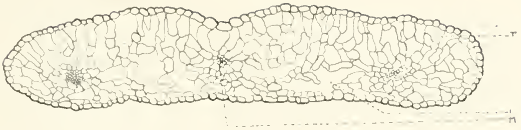
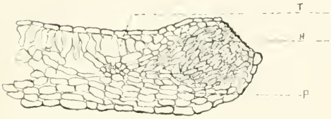


Fig. 6



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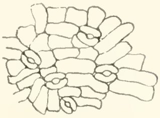

Fig. 8

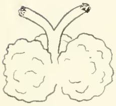

Fig. 12

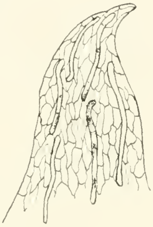

Fig. 13

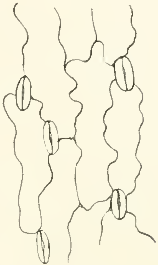

Fig. 9

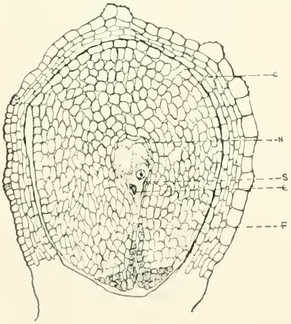

Fig. 11

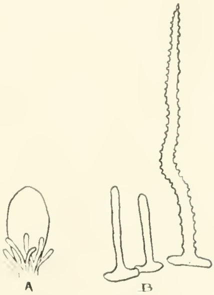

Fig. 10

RUSSELL ON FLOERKEA

BIOCHEMICAL STUDIES OF INSECTIVOROUS PLANTS*

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I. THE WORK OF PREVIOUS INVESTIGATORS ON NEPENTHES

BY

Joseph Samuel Hepburn, A.M., M.S., Ph.D.

Voelcker (1) studied the composition of the pitcher liquor of *Nepenthes*. He describes the liquor from *unopened* pitchers as a clear, colorless liquid with a refreshing taste, an agreeable but not very pronounced odor, and an acid reaction to litmus. The acid was *not* volatilized by evaporation of the liquor to dryness.

The total solids of the liquor were determined by drying at 212° F. (100° C.), the ash by ignition of the solids at a red heat. Total solids are expressed as percent of the pitcher liquor, ash and volatile matter as percent of the total solids. The solids were yellow or cream colored, very hygroscopic, and readily soluble in water. Samples of liquor from different *unopened* pitchers gave the following values:—

Total solids	Volatile matter (loss on ignition)	Ash
1. 0.92		
2. 0.91	25.86	74.14
3. 0.62	36.06	63.94
4. 0.27	32.92	67.08

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* Through the kindness of Dr. John M. Macfarlane, these studies were conducted in the Botanical Laboratory and Nepenthes House of the University of Pennsylvania during 1914-1916. Papers that have appeared since that time have also been cited in the bibliographies.

The liquor from *unopened* pitchers contained potassium, sodium, magnesium, calcium, chlorine (hydrochloric acid), and organic acids; other bases, and sulphuric, phosphoric, oxalic, tartaric, and racemic acids were shown to be absent.

The liquor from *opened* pitchers was yellowish and not always clear; it was acid to litmus, and contained the same acids and bases as were present in the liquor of unopened pitchers. A determination of total solids gave 0.87 percent; the solids were yellow and readily soluble in water. Free volatile acids (including acetic and formic) were absent, for distillation of one-half ounce of the liquor to dryness yielded only distilled water as a distillate.

A further study of the organic acids and the ash was made on the residue obtained by evaporation to dryness of the mixed liquor collected from both unopened and opened pitchers. *The organic acids* were found to consist chiefly of malic acid, plus a little citric acid.

The ash had the following composition:—

Potassium chloride	76.31%
Sodium carbonate	16.44%
Calcium oxide	3.94%
Magnesium oxide	3.94%
Total	100.63%

Using the average value for the volatile matter of the total solids of the liquor of unopened pitchers (see above), and allowing for the carbonic acid content of the ash, the composition of the total solids was found to be:—

Organic matter (chiefly malic acid and a little citric acid)	38.61%
Potassium chloride	50.42%
Sodium oxide	6.36%
Calcium oxide	2.59%
Magnesium oxide	2.59%
Total	100.57%

The yellow color (e.g., of the total solids) is ascribed to a small quantity of "another organic matter."

Hooker (2) found that the pitcher liquor was always acid. The secretion of the liquor was not increased by the introduction of inorganic substances, but was increased by the introduction of animal matter into the pitcher. As substrates, Hooker used fibrin, raw meat, cartilage and cubes of egg white. He reported:—

"After twenty-four hours' immersion, the edges of the cubes of white of egg are eaten away, and the surfaces gelatinised. Fragments of meat are rapidly reduced; and pieces of fibrin weighing several grains dissolve and totally disappear in two or three days. With cartilage the action is most remarkable of all; lumps of this weighing 8 or 10 grains are half gelatinised in twenty-four hours, and in three days the whole mass is greatly diminished, and reduced to a clear transparent jelly. After drying some cartilage in the open air for a week, and placing it in an unopened but fully formed pitcher of *N. Rafflesiana*, it was acted upon similarly and very little slower."

All of the above experiments save the last were apparently conducted in opened pitchers. Experiments, made *in vitro* with pitcher liquor and fibrin, meat, and cartilage, gave a digestion of the substrates "wholly different" from that occurring in the pitchers. Hence the digestive action in the pitchers was not produced by the fluid first secreted by the pitchers.

From these and similar experiments Hooker concluded:—"It would appear probable that a substance acting as pepsine is given off from the inner wall of the pitcher, but chiefly after placing animal matter in the acid fluid; but whether this active agent flows from the glands or from the cellular tissue in which they are imbedded, I have no evidence to show." However, he attributed all three phenomena—secretion of the acid liquor, digestion (secretion of the digestive enzyme), and assimilation—to the glands, and decided that *Nepenthes* possess a true digestive process.

From the following experiments, it seemed probable that the temperature influenced the digestive action. When cartilage or fibrin was kept for six days in pitchers of *N. ampullaria* in a cold room, it was not digested; the substrate, however, was immediately acted upon when transferred to pitchers of *N. Rafflesiana* in a stove house.

If large amounts of substrate were introduced into a pitcher, digestion and absorption did not attain completion, and putrefaction was noted after the lapse of many days.

Tait (3) isolated an enzyme from the liquor of unopened *Nepenthes* pitchers, and also from the liquor of opened pitchers. His procedure was as follows. The liquor was acidulated with dilute phosphoric acid, and a thin suspension of calcium carbonate in water was added until effervescence ceased; the precipitate of calcium phosphate, which adsorbed the enzyme, was permitted to stand for 24 hours, then was separated from the supernatent liquid by decantation, and was dissolved in very dilute hydrochloric acid. A saturated solution of cholesterol in a mixture of absolute alcohol and absolute ether was added to this solution. The precipitated cholesterol, which adsorbed the enzyme, was dissolved in absolute ether. The aqueous layer now contained the enzyme as a gray, amorphous, flocculent, suspended solid, which was partly soluble in distilled water, insoluble in boiling water, very soluble in glycerol, and produced a characteristic viscid change in a small quantity of fresh milk. Unfortunately the power of the enzyme to digest proteins was not tested; and its action on milk recalls that of rennin, rather than that of a true protease.

Tait permitted the liquor from four *virgin* pitchers of *N. phyllamphora* to act for 28 hours on cubes of albumen (volume of each cube 1 cubic millemeter). The substrate remained unchanged. But one sample of the liquor was acid; the other three samples were absolutely neutral; the enzyme described above was found in but one sample.

After the pitchers had opened, the liquor became changed in its reaction, and in its proteolytic power. "Fluid taken from pitchers into which flies have previously found their way is always very acid, has a large quantity of the ferment, and acts in a few hours on cubes of albumen, making them first yellow, then transparent, and finally completely dissolving them." The liquor in a pitcher, in which insects were undergoing digestion, was "very viscid and very acid."

Tait sums up:—"In the unopened pitcher the secretion is only faintly acid and not at all viscid. The secretion is increased therefore, ... , in quality after food has been taken in."

The work of von Gorup and Will (4) was largely carried out on the secretion of *N. phyllamphora*, Willd. and *N. gracilis*, Korth. Separate studies were made, *in vitro*, on (a) liquor from pitchers which were free from insects, and (b) liquor from pitchers which had been entered by insects and contained their remains. These may be spoken of as non-stimulated and stimulated pitchers respectively.

The liquor was almost colorless, faintly opalescent or entirely clear, odorless, without any distinct taste, and of varying consistency—some samples being thick, others being thin like water. The liquor from non-stimulated pitchers was neutral or, at the most, very faintly acid; that from stimulated pitchers imparted a decidedly red color to litmus paper, and the color did not completely vanish on exposure to the air.

Liquor from Stimulated Pitchers

The insect residues were removed by filtration, and the action of the filtrate on certain substrates was studied. Ox-blood *fibrin* was swollen to a gelatinous mass in 0.2 percent hydrochloric acid, then was freed as completely as possible from the adherent acid by means of pressure. A flock of this fibrin was almost completely dissolved by the pitcher liquor in ¾ to 1 hour at a temperature of incubation of 40° C., and in 2 hours at a temperature of incubation of 20° C. The resulting solution was faintly opalescent. The time required for solution of the fibrin was reduced to ¼ hour by addition of several drops of 0.2 percent hydrochloric acid to the liquor. After digestion for two hours, the filtered solution remained clear on boiling, and was not precipitated by mineral acids or by acetic acid plus potassium ferrocyanide, but was precipitated by mercuric chloride, by tannin, and by phosphotungstic acid, and gave an excellent rose-red biuret reaction.

Little slices of *coagulated egg white* were digested with liquor to which 1 or 2 drops of 0.2 percent hydrochloric acid had previously been added. After 24 hours at 20° C., the slices were attacked at the edges, and were transparent; the filtrate from them gave a distinct rose-red biuret reaction.

On digestion at 20° C. with pitcher liquor and 1 or 2 drops of 0.2 percent hydrochloric acid, *raw meat* soon became transparent at the edges and somewhat swollen, and was partly dissolved without putrefaction. A further change was not noted after 48 hours. The filtered solution was not precipitated by boiling, nor by the addition of acetic acid and potassium ferrocyanide, was precipitated by mercuric chloride and by tannin, yielded a cloudy precipitate with phosphotungstic acid soluble in excess of the reagent, and gave a positive biuret reaction.

Legumin became transparent on the edges and somewhat swollen after digestion for 24 hours at 20° C. with pitcher liquor plus 1 or 2 drops of 0.2 percent hydrochloric acid; the filtered solution gave a very decided biuret reaction.

Gelatin, upon which pitcher liquor plus several drops of 0.2 percent hydrochloric acid had been poured, dissolved almost completely after 24 hours at ordinary temperature. The solution was filtered and concentrated to a small volume; it did not gelatinize, but remained a thick syrup; digestion had occurred with the production of gelatin-peptone, and the power to gelatinize had been destroyed.

The pitcher liquor did *not* contain a diastase. The liquor was mixed with a thin starch paste, and then incubated for 24 hours at 20° to 30° C. The starch was not hydrolyzed, the filtered solution was optically inactive, and did not reduce Fehling solution, hence did not contain reducing sugar.

Sufficient liquor from stimulated pitchers was not available to determine the nature of the free acid present in it. It is stated that hydrochloric acid may be excluded.

Liquor from Non-stimulated Pitchers

Gelatinous, swollen fibrin was washed until the acid reaction (due to hydrochloric acid) had almost completely vanished. Flocks of the fibrin, which were placed in the liquor, suffered no noticeable change within several hours at 20° to 30° C., and had not dissolved to the slightest extent at the end of 24 hours; the fibrin had contracted somewhat, and the filtrate from it gave a scarcely noticeable tinge of rose-red in the biuret test. However, the fibrin was almost completely dissolved in 1½ hours by pitcher liquor to which 2 or 3 drops of 0.2 percent hydrochloric acid had previously been added; the resulting solution behaved in every way as did the solution obtained by the action of the acid liquor from stimulated pitchers.

Swollen fibrin, carefully freed from adherent hydrochloric acid, was dissolved almost instantaneously at the ordinary temperature by liquor to which 3 or 4 drops of dilute formic acid had previously been added. The residue, which was scarcely noticeable, was removed by filtration, and the filtrate was carefully neutralized. The very slight precipitate which formed was collected on a filter; the filtrate gave none of the reactions of the true proteins, but did give an intense biuret reaction. When the formic acid was replaced by acetic acid, or by propionic acid, the fibrin was digested less rapidly, the rate of digestion now being about the same as in the liquor of the stimulated pitchers. At a temperature of 20° to 30° C., the fibrin was completely dissolved in 2 to 3 hours; the resulting solution contained mainly metaproteins and gave a very faint biuret reaction.

When the pitcher liquor was previously acidified with malic acid, the fibrin was almost completely dissolved in 10 minutes at ordinary temperature; the resulting solution gave a faint but distinct biuret reaction. After the digestion had been in progress for 2 hours, the precipitate on neutralization was very slight, and the biuret reaction decidedly more marked. When citric acid was substituted for malic acid, the fibrin was dissolved in a considerably shorter time; after digestion for 2 hours, the resulting solution gave a very slight precipitate on neutralization, and gave a biuret reaction as intense as that obtained in the case of formic acid.

The solutions, obtained by digestion, contained much more protein (metaprotein) immediately after the fibrin had dissolved, than in later stages of the digestion. This protein gradually vanished, and was displaced by peptone as the period of digestion lengthened. From these phenomena, which were repeatedly observed, it followed that, apparently, peptone represented the second and not the first stage of the action of the enzyme.

Von Gorup and Will, who made the necessary control experiments on their reagents, do not hesitate distinctly to designate the acid liquor of the stimulated *Nepenthes* pitchers as a solution of plant pepsin. The neutral secretion of the non-stimulated pitcher, like pepsin in the absence of free acid, exerts no digestive action.

Vines (5) dehydrated pitchers of *Nepenthes* species (*N. hybrida* and *N. gracilis*) by means of absolute alcohol, converted their tissue into a pulp, and extracted this with glycerol in order to obtain a solution of the protease. As a substrate, he used fibrin which had been soaked in 0.2 percent hydrochloric acid until gelatinous. When the fibrin was incubated at 40° C. with the enzyme solution to which a few drops of 0.2 percent hydrochloric acid had previously been added, at the end of 8 hours the substrate showed signs of digestion and, after filtration, the solution gave a distinct peptone (biuret) reaction. Control experiments on fibrin plus enzyme solution, and on fibrin plus 0.2 percent hydrochloric acid, did not show digestion and did not give a biuret reaction. Hence the glands of the pitchers contained a proteolytic enzyme, which was soluble in glycerol and was active only in the presence of acid.

Pitchers were gathered from the same plants (of the species mentioned above) at the same time. Some pitchers were immediately treated as outlined above for the preparation of a glycerol extract. Other pitchers were first treated with dilute (1 percent) acetic acid for 24 hours, then the glycerol extract was prepared, using the procedure already described. In every set of experiments, the extract from the pitchers, which received the preliminary treatment with acetic acid, was higher in proteolytic power than the extract from pitchers not so treated. Thus the same volume of each extract was permitted to act on a pellet of swollen fibrin in the presence of 2 cc. of 0.2 percent hydrochloric acid at a temperature of 40° C. The fibrin pellets were similar. At the end of 6 hours, the extract from pitchers, which had received the acid treatment, had completely dissolved the fibrin, while the extract from pitchers not so treated had but slightly attacked the fibrin. The solutions, obtained by filtration of the contents of each tube, always gave a positive response to the biuret test.

Vines states:—"These experiments seem to indicate that in the gland-cells of the *Nepenthes* pitchers, ..., the digestive ferment exists at first in combination with some other body, as zymogen—and that in plants, as in animals, this zymogen can be split up by the action of dilute acid, the free ferment making its appearance as a result of this decomposition."

In another report on this research, Vines (25) also described tests for the presence of diastase in the pitchers. The glycerol extracts of the pitchers were without action on starch, therefore, did not contain diastase. He also noted that the glycerol extract did not contain sugar.

During a further study of the proteolytic enzyme of *Nepenthes*, Vines (6) found that pitcher liquor plus 0.25 percent hydrochloric acid completely digested fibrin in the presence of bactericides such as potassium cyanide, chloroform, thymol. The fibrin was dissolved when mercuric chloride (approximately 0.5 percent) was used as a bactericide; however, the proteolysis was somewhat retarded. Fibrin was also digested by pitcher liquor to which had been added 1 drop of concentrated hydrochloric acid and sufficient hydrocyanic acid to render the concentration of the latter acid one percent. The liquor plus 0.25 percent hydrochloric acid partially digested coagulated egg albumen in the presence of potassium cyanide or thymol, the products of proteolysis being detected by the biuret test.

The enzyme and its proteolytic power were destroyed by the action of 1 percent sodium hydroxide for 1 hour, or of 5 percent sodium carbonate for three hours, at a temperature of 35 to 40° C.; the solutions were then neutralized, and tested for protease in the usual way, but digestion of the substrate never occurred.

The influence of the following concentrations of hydrochloric acid on the proteolysis was studied:—1%, 0.5%, 0.25%, 0.125%. The optimum acidity for digestion was found to be 0.25%.

The enzyme was isolated from 100 cc. of pitcher liquor by the following procedure. An equal volume of absolute alcohol was added, then phosphoric acid and lime water; the solution was neutralized with ammonium carbonate; the precipitate was collected on a filter, and permitted to drain over night. A portion of the precipitate was shaken with 10 cc. of 0.25 percent hydrochloric acid, and the solution was filtered; the filtrate digested fibrin, while a control experiment on fibrin plus 0.25 percent hydrochloric acid gave no digestion. After the precipitate had been kept in a bottle with chloroform vapor for a month, the enzyme contained in it was still active.

Vines was unable to find a zymogen of the protease in the pitcher liquor, although the activation of a zymogen was suggested by the requirement that acid be added in order to produce proteolysis.

Glycerol extracts, prepared from the washed and dried tissue of relatively young, vigorous pitchers, contained a protease which acted in the presence of 0.25 percent hydrochloric acid, and retained its activity for as long as 2 months, but not indefinitely.

Vines concluded that the digestive action of the pitcher liquor is due to an enzyme. He detected albumose, but not peptone, as a product of the digestion, and suggests that peptone was formed and immediately split into other compounds. Wheat gluten was digested in the same manner as fibrin.

Most of these experiments were made on *N. mastersiana*. The liquor in the unopened pitchers of this species usually was distinctly acid.

In his next paper on the proteolytic enzyme of *Nepenthes*, Vines (7) studied the action of heat on the protease. The pitcher liquor was heated, then cooled; acid and fibrin were added, and digestion was carried out as in the previous study. Control experiments were made on the unheated pitcher liquor. Heating the liquor at 80° C. for 15 to 20 minutes did not destroy the enzyme, but the proteolytic power was decreased to a marked degree. Thus in one experiment the heated liquor completely digested the fibrin in approximately 4 days, while the unheated liquor in the control experiment required but 3 hours to digest the fibrin completely. When the liquor was held at 78° to 83° C. for 30 minutes, the enzyme was completely inactivated; no digestion occurred in 4 days, while the control experiment on unheated liquor gave complete digestion of the fibrin in 1½ hours. When the liquor was kept at 80° C. for 30 minutes, the enzyme was completely inactivated, and exerted no proteolytic action on fibrin after digestion for 1 week; the unheated control completely digested the fibrin in 5 hours. Boiling the liquor "for some seconds" partly inactivated but did not completely destroy the enzyme. It was necessary to subject the liquor to a temperature of 100° C. "for an appreciable time, say 3-5 minutes," in order to destroy the enzyme completely.

The destructive action of sodium carbonate on the enzyme was also studied. The degree of inactivation of the enzyme depended on the concentration of the alkali, the period of time during which it acted, and the temperature at which it acted. Sufficient solid sodium carbonate was added to the pitcher liquor to bring the salt to the desired concentration. After the resulting solution had been incubated at the desired temperature for the desired period of time, it was neutralized, then acidified with hydrochloric acid, and a digestion experiment was made in the usual way to determine the enzyme activity. The concentration of the sodium carbonate varied between 0.5 and 5 percent; it was permitted to act on the enzyme at a temperature of either 35° to 38° C. or 50° C. for a period of time varying between 30 minutes and 17 hours. Each control experiment on untreated liquor was incubated at the same temperature and for the same period of time as its determination proper; then its enzymic activity was determined.

The typical series of experiments in the following table may be quoted. The substrate in each experiment was 0.01 gram of fibrin.

Series	% of sodium carbonate	Time of incubation with sodium carbonate at 50° C.	Proteolytic activity subsequently
a	5	1½ hours	no digestion in 6 days
	1	1½ hours	digestion complete in 4 days
	Control		digestion complete in 3½ hours
b	5	1½ hours	no digestion in 5 days
	1	1½ hours	no digestion in 5 days
	Control		digestion complete in a few hours
c	1	1 hour	no digestion in 4 days
	Control		digestion complete in a few hours

"On comparing the results of *a*, *b*, and *c*, it would appear that treatment with 1% Na_2CO_3 for one hour at a temperature of 50° C. is an approximate index to the stability of the enzyme."

The pitcher liquor lost its acid reaction and its coloration, when passed through a Berkefeldt filter. "It still retains some digestive power, but is far less active than unfiltered liquid, the period of digestion being more than doubled." Filtration through a Berkefeldt filter caused a marked loss of the enzymic power of solutions of pepsin (from the stomach of the pig) and of salivary ptyalin. Hence the partial loss of proteolytic power by the pitcher liquor was due to the retention by the filter of a true proteolytic enzyme, and not to the retention of proteolytic bacteria, *i.e.*, the liquor owed its proteolytic power to a true enzyme.

Experiments (usually, though not invariably, made on washed, unopened pitchers) showed that "under certain circumstances, previous treament with acid causes the glands of the pitcher to yield a more active glycerin-extract, or to yield an active extract when otherwise the extract would be inactive; and it can only be concluded that this must be due to the presence of a zymogen in the glands from which the enzyme is liberated on treatment with acid." The zymogen was best converted into active enzyme by treatment of the glandular tissue with acid for a short time at a relatively high temperature, say ¾ hour at 50° C. Action of the acid for a longer time at lower temperature, not only activated the enzyme but also extracted it from the glands. In the activation experiments, 0.25 percent hydrochloric acid was used; 0.5 percent acetic acid apparently was less satisfactory. One function of the high acidity of the liquor in unopened pitchers probably is to activate the zymogen.

Peptone and leucine were recognized among the products of digestion.

Vines concludes that the enzyme is derived from a zymogen which is present in the gland cells. The enzyme is a tryptic ferment, requires an acid medium for its action, and resembles the proteases of germinating seeds with respect to the reaction of the medium, which it requires, and the products, which it forms.

In his final paper on the proteolytic enzyme of *Nepenthes*, Vines (8) applies to it the name "Nepenthin." Fibrin and Witte peptone were subjected to a somewhat prolonged digestion at 38.5° C. with pitcher liquor, to which hydrochloric or citric acid had been added. The following are typical experiments:—

I. 10 grams moist fibrin
50 cc. 0.3% hydrochloric acid
50 cc. pitcher liquor (from *N. Mastersiana*)
Period of incubation 18½ hours

II. 1 gram Witte peptone
0.2 gram citric acid
10 cc. distilled water
40 cc. pitcher liquor
Period of incubation—from noon until morning of the next day.

After digestion, the resulting solutions gave the tryptophane reaction (a violet or pink color with chlorine water), which is stated to be characteristic of tryptic digestion only.

The solutions, obtained by digestion, were evaporated to dryness, the residue was extracted with absolute alcohol, and the alcohol was removed by evaporation. This residue gave a biuret reaction. Hence tests could not be made for *free* tyrosin among the products of the proteolysis, for the tyrosin reactions would have been given by the combined tyrosin groups of the albumose and peptone, which were present in the residue from the alcoholic solution.

Dubois (9) studied the pitcher liquor of the following species of *Nepenthes:—coccinea, distillatoria, Hookeriana, hybrida, maculata, phyllamphora, Rafflesiana.* Before the opening of the operculum, the pitcher liquor of all these species was limpid, slightly viscid and slightly acid. In opened pitchers, the liquor generally was thick, contained whole insects, and, at times, emitted a strong odor of putrefaction.

When liquor was removed from closed pitchers, which were about to open, and was immediately placed in contact with cubes of coagulated albumen, then incubated at the temperature of the atmosphere, or at a temperature of 35° to 40° C., the albumen was not attacked; the liquor remained limpid at the end of several hours. It was then filtered; the filtrate contained no peptone. The experiment was repeated by transferring the liquor from closed pitchers to Pasteur culture tubes which contained albumen cubes. The results were the same as before; the angles of the cubes remained absolutely intact, and neither micro-organisms nor putrefaction were present at the end of several days.

Liquor from pitchers, which had been open for but a short time, was still clear. However, it attacked cubes of egg-white, quite rapidly at ordinary temperatures, and very rapidly at the temperature of the incubator. The cubes became swollen, transparent and gelatinous, and lost their angles; the liquor became viscid, and a distinct odor of putrefaction developed in some of the tubes. The liquor contained numerous micro-organisms of different species and, after filtration, gave some of the reactions of peptone. Many open pitchers contained insects, which were in process of putrefaction and not in process of digestion.

The manner in which coagulated egg albumen behaved in the presence of the pitcher liquor—contaminated or not contaminated by micro-organisms—led Dubois to the following conclusions:—

(1) That the liquor does not contain any digestive substance (enzyme) comparable to pepsin, and that *Nepenthes* are not carnivorous plants.

(2) That the phenomena of disintegration or pseudo-digestion, observed by Hooker, were due without any doubt to the activity of micro-

organisms which came from without the pitcher, and were not due to a secretion of the plant.

Couvreur (26) supported the conclusions of Dubois, and maintained that the digestive phenomena observed by Vines were due to the action of the reagents on each other, and not to the presence of a protease in the pitcher liquor of *Nepenthes.*

Tischutkin (27), in a paper on *Pinguicula*, commented on the work of Von Gorup and Will. He considered that the protease, which these investigators found in the pitcher liquor of *Nepenthes*, was entirely of bacterial origin.

Among the insectivorous plants studied by Tischutkin (10) was *Nepenthes Mastersi.* The pitchers were stimulated by means of small, sterile cubes of albumen. Even 24 hours later, the liquor of the pitchers contained myriads of bacteria, as was regularly shown by the direct microscopic examination. The bacteria were isolated by cultures on weakly acid nutrient gelatin and were tested for their peptonizing power; several species were always found which dissolved small, sterile albumen cubes fairly rapidly in an acidified menstruum.

The following experiments were also carried out. Incisions were made in the side wall of pitchers, which had not yet opened and therefore contained no bacteria; the liquor was removed with a pipette and conveyed to test glasses which contained water and small cubes of albumen (1 cube in each glass); in some glasses the water was neutral, in other glasses it had been acidfied. The experiments were carried out with antiseptic precautions. The results showed that the pitcher liquor did not contain a peptonizing enzyme, for the substrate remained unchanged after incubation for 48 hours at 37.5° C. In order to overcome the possible objection that the pitchers had been too young, the experiments were repeated in a modified form. Openings were made in the wall of pitchers, which had not yet opened; small, sterile cubes of albumen were introduced into the cavities of the pitchers; the openings were closed; and the plants were permitted to remain undisturbed. When the pitchers opened, 4 days later, the albumen cubes were found unaltered; their angles had *not* been rounded off; the liquor had a strongly acid reaction and contained no peptone; and bacteria were present "in very slight number." When the liquor of these pitchers was placed in test glasses and treated anew with small cubes of albumen, it dissolved the cubes only after 4 to 5 days had passed, *i.e.*, at a time when the bacteria had already multiplied to a considerable degree.

Tischutkin maintained that the digestion of protein in the liquor of insectivorous plants is produced exclusively by the vital activity of micro-organisms, which are always present in the secretion of the fully developed plant, entering from the air and also with the bodies of the insects, etc. The rôle of the insectivorous plants is therefore limited; they secrete a medium favorable for the activity of the peptonizing micro-organisms, and they make use of the products of this activity.

Goebel (11) made an elaborate research on the pitcher liquor. Pitchers of *Nepenthes paradisiaca* (a hybrid) contained a clear, colorless, tasteless fluid free from insects. Fibrin flocks were placed in the pitchers, and also in water as a control. In both cases, after 6 days, the flocks had been disintegrated into little shreds, and innumerable bacteria were present. The liquor, in all the experiments, was either neutral or very faintly alkaline, and contained no peptone. The liquor from the pitchers gave no color with Nessler's reagent; the solution from the flasks, which contained water and fibrin, gave a strong yellow color with this reagent. Hence, in the pitchers, the nitrogenous compounds produced by the proteolysis of the fibrin were absorbed, either as ammonia or as some other compound.

Liquor was removed from the pitchers, in which the fibrin had been digested, and was sown on nutrient gelatin. The gelatin became liquefied to a marked degree in 2 days and acquired a green fluorescence which is characteristic of *Bacillus fluorescens liquefaciens*. Neither bacteria nor moulds were found when liquor from unopened pitchers was inoculated into the gelatin.

Liquor was collected from unopened pitchers of *N. paradisiaca*; it exerted very little digestive action after 0.2 percent hydrochloric acid had been added, forming very little peptone; therefore very little enzyme was present.

The absorption of ammonia by the pitcher was demonstrated by the following procedure. A definite volume of aqueous solution of ammonia (containing 1 part of ammonia in 20,000 parts of solution) was placed in a pitcher, an equal volume of the solution was placed in a glass vessel in the *Nepenthes* house as a control. Twenty-four hours later, the volume of liquid within the pitcher was practically unchanged. However, Nessler's reagent produced no precipitate with the contents of the pitcher, and formed a thick precipitate with the control experiment; therefore ammonia had been absorbed by the pitcher. Further experiments demonstrated that one-half of the ammonia was absorbed during the first three hours, and that all the ammonia had been absorbed at the end of 6 hours; in these experiments Nessler's reagent was used for the colorimetric determination of ammonia.

Pieces of meat, the size of grains of rye, were introduced into pitchers; after 2 days, the contents of 3 pitchers were acid in reaction, those of 5 pitchers neutral in reaction; after another week had passed, the contents of but 1 pitcher had an acid reaction which, however, was not very marked.

A vigorous plant of *Nepenthes paradisiaca* was cultivated in a glass chamber at a temperature of 20° to 25° C. in an atmosphere saturated with moisture. The plant bore 3 pitchers. The oldest of the pitchers was brownish and no longer vigorous, and contained a small amount of neutral liquor. A wasp was introduced into the liquor and soon died; 3 days later the liquor had an alkaline reaction; bacteria and infusoria were present in abundance. The second pitcher contained an acid liquor, in which a small fly was present. This liquor dissolved fibrin (which had been stored in glycerol, washed, and strongly swollen) in 1 hour; after 3 hours, soluble protein was absent, but peptone was present; the temperature of incubation was 25° C. Another fibrin flock and 0.2 percent hydrochloric acid were added, and the temperature of incubation was changed to 16° to 18° C.; the flock dissolved in 40 minutes; this solution was sown on nutrient gelatin, and bacteria were not found.

The youngest pitcher was still closed. Inoculation of its contents into nutrient gelatin gave no growth. The liquor was neutral in reaction and mucilaginous. A flock of swollen fibrin and 0.1 percent formic acid were added to the liquor; the flock was completely digested in 12 hours; even after 8 days, bacteria were absent as was shown by a nutrient gelatin culture, which was made in duplicate. It was also found that 0.1 percent formic acid prevented the development of putrefactive bacteria in an approximately 0.5 percent peptone solution, which was exposed in the open air for 8 days, and that only a few mold-threads developed. When the acid had not been added to the peptone solution, clouding and an unpleasant odor soon appeared, due to the development of innumerable bacteria.

Goebel interprets these observations:—

"These facts in themselves suffice to refute the acceptance of a bacterial digestion. They show that, in the *Nepenthes* hybrid studied, even in the unopened pitcher, a peptonizing enzyme is present which exerts an energetic digestive action on the addition of acid. Normal pitchers, into which an insect falls, very soon secrete formic acid. With excessive feeding, of course, the enzyme action is insufficient and putrefaction may occur even in otherwise normal pitchers; it is probable, but not certain, that an increased secretion of enzyme occurs in the opened pitchers in the presence of stimulating substances." However, an increased secretion could not be detected in a pitcher stimulated by a fibrin flock, and compared with an unstimulated pitcher as a control; but one such experiment was made.

The tests for a protease in the glands of the pitcher-cover, which secrete nectar, were entirely negative. Flocks of fibrin were fastened over the glands by means of filter paper, which was kept moist. Digestion of the fibrin did not occur.

The glycerol extract of the pitchers contained too little enzyme and showed no proteolytic activity. Apparently the procedure of Vines (5) for the detection of enzymic activity was followed.

The secretion of unopened pitchers of *Nepenthes paradisiaca* was neutral in reaction. The liquor of unopened pitchers of *Nepenthes Mastersiana* was strongly acid; when fibrin was introduced into these pitchers, it was dissolved in 3 days, and cubes of albumen were strongly attacked; the protein was peptonized in the pitcher, and no bacteria were present.

A pitcher of *Nepenthes Sedeni* contained a strongly acid liquor and dissolved fibrin in 25 hours. Similar observations were made with a pitcher of *Nepenthes robusta*. Cultures demonstrated the absence of bacteria. The filtered solutions from the pitchers plus 0.1 percent formic acid digested meat fibres in 5 hours at a temperature of 35° C.

Goebel states that free formic acid could be shown to be present in the secretion (pitcher liquor) of the species of *Nepenthes* studied by himself. The total acidity of different pitchers, calculated as formic acid, was:—0.036%, 0.025%, 0.021%. All bacteria are not killed by this degree of acidity.

The strongly acid pitcher-liquor of *Nepenthes Mastersiana* was sown on non-acid nutrient gelatin, and on nutrient gelatin rendered acid by the addition of 0.2 percent tartaric acid. After 8 or 9 days, no growth was noted, or else a few bacteria and molds, which were doubtless due to air-contamination.

Goebel considered that a true enzymic digestion occurred in normal pitchers of *Nepenthes*, in which the liquor had not been diluted by water; this dilution often occurs in greenhouses. The enzyme was classified as a peptonizing enzyme, not identical with pepsin, and different from the pancreatic protease. Bacterial digestion could occur only in the liquor of enfeebled pitchers, which possessed a neutral or alkaline reaction; it was recorded as much slower than the normal digestion, although its products could be absorbed, in part at least, by the plant, provided the putrefaction had not injured the pitcher. Goebel stated that the pitchers of enfeebled plants frequently contain only water, and that such plants may give results like those obtained by Dubois and by Tischutkin.

The absorption of peptone by the pitchers was demonstrated. Six pitchers of *N. Mastersiana* were washed out; and a known volume of a 0.1 percent solution of peptone, containing 0.1 percent formic acid, was placed in them. At the end of 66 hours, the pitchers were emptied. Resorption and evaporation of the liquid had been but slight, it was clear, colorless, free from bacteria, and contained only a trace of the mycelium of mold. The formic acid content had not decreased; however, but a faint trace of peptone remained, and an enzyme could not be detected. Hence the peptone had been absorbed. These pitchers again secreted liquor, and digested small pieces of meat.

Clautriau (12) conducted experiments on plants of *Nepenthes melamphora* in their native habitat in Java. The liquor in non-stimulated pitchers was neutral to litmus. When an unopened pitcher was merely shaken, its liquor had a more or less acid reaction on the following day. The reaction became acid after fine glass tubes, 1 to 2 cm. in length, were dropped through the lid into the pitcher, or after 2 or 3 drops of tincture of litmus were added to the liquor within the pitcher. Hence slight stimulation sufficed to cause the appearance of acid. The strongest acid reaction in the stimulated pitchers was about equal to that possessed by a solution, obtained by diluting 2 cc. of slightly fuming hydrochloric acid with sufficient water to render the final volume 1 liter. The liquor contained in solution a substance which was apparently thermolabile; this substance caused insects to become wet and to sink. The liquor did not contain a poison which kills insects. The insects were finally digested, leaving only a residue of chitin; putrefaction did not occur. The pitchers were sensitive to the presence of even exceedingly small quantities of antiseptics, such as formaldehyde, chloroform, spirit of camphor, essence of peppermint and of lemon, in the pitcher liquor; these reagents caused a cessation of the secretion of acid and of digestion, and the pitchers died in a few days.

When coagulated egg-white was introduced into the pitchers, digestion and absorption occurred. If but a small quantity of egg-white was used, absorption equalled digestion; proteolytic products did not remain in the pitcher, and a substrate for bacteria was lacking. If a large quantity of egg-white was used, the unabsorbed products of digestion accumulated, and the pitcher was invaded by bacteria.

When a sterile solution of egg-white, prepared as follows, was introduced into the pitchers, digestion occurred; and bacterial invasion and putrefaction were rarely noted. Ten cc. of white of egg and 90 cc. of water were shaken together, to break up the membranes in the white and to dissolve the albumin. The solution was filtered and 0.1 milligram of ferrous sulphate was added, *i.e.*, 2 drops of a freshly prepared, 0.1 percent solution of ferrous sulphate. If the egg had not been fresh, more iron was added, but never an amount in excess of 1 milligram of ferrous sulphate. The solution was then boiled; it usually remained clear and limpid, but at times showed a faint opalescence. After conducting a digestion experiment with this solution as the substrate, the undigested albumin was removed by the following procedure. An alkaline salt was added to the solution, which was then acidified very quickly, and the albumen was coagulated with heat.

In most vigorous pitchers, all the protein was digested at the end of two days. Thus 5 cc. of the solution of egg-white, described above, was introduced into a pitcher. At the end of two days the pitcher liquor gave no precipitate on neutralization, and on boiling in the presence of salts or of acids; it yielded merely a trace of a precipitate with the following reagents:—potassium mercuric iodide, acetic acid and potassium ferrocyanide, phosphomolybdic acid.

The plant itself played an important rôle in the digestion. Experiments were made *in vitro* with liquor from both unopened and open pitchers, using chloroform as a bactericide. Absolutely no digestion of the substrate occurred. In one experiment, liquor, which possessed digestive power while in the pitcher, digested the substrate *in vitro*; the albumen disappeared, and much albumose (proteose) and possibly a little peptone were formed. Separation of the pitcher from the plant during the course of digestion inhibited the digestion of the albumin.

Clautriau also conducted experiments at Brussels on *Nepenthes* which had been cultivated in greenhouses.

He used the following technique in studying the products of the proteolysis. After digestion, the solution was neutralized with dilute sodium hydroxide solution in order to separate the syntonin (acid metaprotein), which was collected on a filter. To the filtrate were added an equal volume of saturated solution of sodium chloride and a trace of acetic acid; the solution was boiled to coagulate the albumin, which was then removed by filtration. The filtrate was saturated, while hot, with ammonium sulphate, first while acid, then while alkaline in reaction; the resulting solution was permitted to stand; and the albumoses (proteoses) collected, and were removed by filtration. In the filtrate, the ammonium sulphate was removed by means of barium carbonate, and the excess of barium was removed with sulphuric acid. The solution was then filtered and concentrated; it contained no albumose, for it did not form a precipitate with potassium mercuric iodide, or with acetic acid and potassium ferrocyanide; it contained peptone, for it responded to the biuret test, and gave precipitates with tannin, phosphotungstic acid, and phosphomolybdic acid.

The liquor in a pitcher of *Nepenthes Mastersiana*, containing insects, was filtered and used in digestion experiments *in vitro*. Three cc. of the liquor and 20 drops of albumin (egg-white) solution, prepared as described above, were permitted to react for 3 days, with and without the addition of 1 drop of hydrochloric acid (1 drop contained 0.01 gram of hydrochloric acid), in the presence of camphor as a bactericide. The albumin was completely digested to peptone in both the presence and the absence of the hydrochloric acid, while a blank experiment, heated for 10 minutes at 100° C. at the beginning of the experiment, contained no peptone. The digestion *in vitro* by the liquor plus hydrochloric acid was far more rapid at 37° C. than at 20° C.

Experiments were made *in vitro* with the liquor of non-stimulated (unopened) pitchers of *Nepenthes coccinea* and of a *Nepenthes* similar to *N. phyllamphora*. Albumin was the substrate, and hydrochloric acid was added. After digestion in the incubator for 5 or 6 days, syntonin and a little albumose were present, but no peptone, and enzyme action probably had not taken place. However, Clautriau hesitated to advance the proposition that the secretion of the enzyme, like that of the acid, is the result of stimulation, although the two experiments were concordant.

The products of proteolysis were rapidly absorbed by the pitcher. Successive and abundant additions of albumin were supported perfectly, without inconvenience. In four days, the pitcher of *Nepenthes Mastersiana*, mentioned above, digested 2.5 cc. of albumin solution, and completely absorbed the products. Then 10 cc. of albumin solution (nitrogen content determined by the Kjeldahl method as ammonia equalled 14 cc. of 0.1 *N* sulphuric acid) were introduced into the pitcher; 7 days later the liquor contained nitrogen equal to but 2.8 cc. of 0.1 *N* sulphuric acid. Another 10 cc. portion of albumin solution was added; at the end of 7 days the nitrogen content of the liquor equalled but 2.7 cc. of 0.1 *N* sulphuric acid. For the third time, 10 cc. of albumin solution were placed in the pitcher, and its protein was also digested. It should be noted that a portion of the nitrogen, found in the liquor after the albumin had been digesting for a week, was due to the enzyme and to the chitin of insects.

Digestion also took place in the pitcher of *Nepenthes coccinea*. In but two instances was peptone found in the pitchers after digestion of albumin, once with a plant of *Nepenthes coccinea*, and once with a plant of *Nepenthes* from Borneo similar to *N. phyllamphora*. The peptone, it is stated, is diffusible, and probably is absorbed.

Clautriau classified the protease of the pitcher liquor as a pepsin, since it acted only in an acid medium, and formed true peptone as the ultimate product of its action. Leucine, tyrosin, and other crystalline compounds were not found among the products of proteolysis.

An amber color, becoming red with alkali, was very frequently noted in the liquor after digestion. It was ascribed to the presence of tannin derived from the glands, and not to the presence of tryptophan.

The pitcher liquor did not contain an amylase, for it had no action on starch paste, when the mixture of liquor and paste was digested for 5 days.

Stimulation was found necessary to excite an *abundant* secretion of both the acid and the protease. The glands, which secrete both the acid and the enzyme, are said to absorb the digested protein. The microchemical reactions of the proteins were more intense in the region of the glands, hence it was concluded that the peptone was absorbed by the glands and stored as protein.

Since the liquor, obtained from healthy pitchers of *Nepenthes melamphora* in its natural habitat, failed to produce proteolysis *in vitro*, it was suggested by Clautriau that the pitchers of this variety possibly absorb either albumin or albumose. He also pointed out that, if too many insects accumulate in a pitcher, they may be decomposed by bacteria without injury to the plant, which probably utilizes the ammonia and amino acids formed by the bacteria.

Clautriau looked on the digestion in the pitcher as a source of nitrogen, and possibly of mineral food, for the nutrition of the plant.

Fenner (13) used *Nepenthes Rafflesiana* Jack in his study of the proteolysis in the pitchers of *Nepenthes*. He states that the innumerable glands of the pitcher lie in niches which open downwards, and are from one-half to two-thirds covered by a projecting, roof-like, epidermal structure. Wet insects, climbing up the wall to escape from the pitcher, come under the "roof" of a gland, and at the same time come in contact with the gland itself, and adhere to it. The gland now secretes a slight quantity of a sticky mucilage, which is more viscous than the other secretion of the glands—the pitcher liquor, that is found even in unopened pitchers. The mucilage dissolves that part of the insect-body which can be utilized by the plant. When the surface of the gland becomes dry by absorption, the undissolved insect-residue falls from the gland, and is usually washed down by the pitcher liquor, either as its level rises or through the swinging motion of the pitcher, or its leaf. The resulting sediment consists for the most part of chitin. The glands on the border of the level of the pitcher liquor come in contact with the insects in this manner, and the cells of these glands show aggregation-phenomena in consequence of the absorption of organic substances, while the cells of glands above the level of the liquor, or beneath its level and merely washed by it, usually have unclouded contents.

When a number of gnats were placed in a pitcher, which was just opening, they swam on the surface of the liquor and came in contact with the glands at its level. The pitcher was emptied, a portion of the glandular region was dried, and some gnats were placed on the dry place; the secretion occurred only after the course of 4 to 6 hours, was slight in amount and insufficient to digest the insects, but dried up about them. When an insect was wet with the pitcher liquor and then brought on the dry place in the glandular region, very soon secretion of the mucilage, digestion, and absorption took place, so that only the residue of chitin was left at the end of 5 to 8 hours. The liquor, used for wetting the insect, must not have been diluted by water; since water may enter the pitcher during the watering of the plant, the liquor should be taken from a pitcher from which water has been excluded by means of a cotton plug.

Greenhouse plants are abnormal in that they usually contain only a slight quantity of liquor, secreted by the glands; as a consequence the greatest portion of the glandular region is not wet by the liquor. Fenner used suitable control experiments in his research.

From his own experiments, and from the observations of Goebel(11) (see above), Fenner draws the following conclusions:—

(1) Normal pitchers, in which insects are found, contain a faintly acid liquor (formic acid according to Goebel), which acts upon the glands as a chemical stimulant when insects, wet with it, come in contact with the glands.

(2) The liquor thus gives rise to digestion, so that insect bodies, which are saturated with it, can rapidly be dissolved and absorbed if they come

upon the glands of the pitcher wall beneath the roof-like epidermal structures.

(3) Insects, which enter the pitcher liquor, can be completely dissolved with the exception of their chitin plates; the latter are found as a sediment.

Fenner considered it a question for further study whether (a) the dissolved products of the digestion were withdrawn from the liquor and absorbed by the glands, or (b) their solution in the liquor was absorbed as such by the glands. Apparently the same glands, that secrete the pitcher liquor and the digestive mucilage, absorb the products of digestion.

Abderhalden and Teruuchi (28) studied the action of the pitcher liquor of *Nepenthes* on the dipeptide glycyl-*l*-tyrosin. This peptide is quite soluble in water, and is not cleaved by pepsin-hydrochloric-acid but is readily split by trypsin into its components, glycine (glycocoll) and *l*-tyrosin; the latter compound is very difficultly soluble in water, and precipitates.

Liquor was obtained from pitchers with closed lids, and from open pitchers which appeared to contain no very large quantity of condensation water. It was viscous, neutral in reaction, and exerted a very slow but distinct proteolytic action on fibrin flocks; after digestion for 3 days, the fluid was removed by filtration and then gave a distinct biuret reaction.

One gram of glycyl-*l*-tyrosin was dissolved in 10 cc. of liquor, collected from several pitchers, and toluene was added as a bactericide. The solution was kept at room temperature for 7 days; a slight cloudiness occurred, but no precipitate formed. Then the solution was transferred to an incubator; the opalescence did not increase; and a precipitate did not separate, even when the solution was evaporated to half its original volume.

The conclusion was drawn that the protease of *Nepenthes* is not a trypsin. "It therefore seems that the flesh-eating plants, *Nepenthes*, do not act through a trypsin-like enzyme. We do not venture to record our finding as a certainty, since from lack of material, it was not possible for us to repeat the experiment under different conditions."

Robinson (14) introduced various solutions into pitchers of *Nepenthes distillatoria* and noted their action upon the pitchers. A dilute (M/1024) solution of potassium nitrate had exerted no injurious action at the end of 9 days, but the pitcher began to wither at the end of 12 days. The nutrient solution of Sachs (calcium nitrate 6 grams, potassium nitrate

1.5 grams, dipotassium phosphate 1.5 grams, magnesium sulphate 1.5 grams, ferrous sulphate a trace, water 6000 cc.) caused a withering of the tissues on about the eighth day. When a dilute solution of Liebig's meat extract was introduced, and the plants then observed for a period of two weeks, the contents of the pitchers did not become foul, and the pitchers did not decay. A 10 percent solution of glucose, kept in the pitchers for 4 days, apparently had no harmful effect on the plant; the solution retained its power to reduce Fehling solution.

Robinson also made tests concerning the secretion of enzymes by the pitchers. A 10 percent solution of sucrose, which had been in the pitchers for 4 days, failed to reduce Fehling solution, whence it was inferred that an invertase is not secreted by the pitchers. A thin starch paste was kept in the pitchers for 4 days; it then had no reducing action on Fehling solution, and "the iodine test showed that the starch granules in the paste had not been broken down"; therefore the pitchers do not secrete an amylase (diastase).

Neutral olive oil and water were mixed in the proportion of 0.4 cc. of oil and 9.6 c.c. of water. After the mixture had been thoroughly shaken, it was introduced into pitchers, and permitted to remain in them for a period of from 4 to 7 days, then removed and titrated with 0.01 *N* potassium hydroxide solution, using phenolphthalein as an indicator. Control experiments were carried out (a) with the emulsion of oil *in vitro*, and (b) with the emulsion plus toluol in the pitchers. The results indicated that hydrolysis of the oil did not occur, and that the pitcher does not secrete lipase. Tap water, which had been kept in the pitchers for 1 day, was used in an experiment with ethyl butyrate; 2 cc. of this water and 4 drops of the ester were allowed to react at room temperature for 24 hours; the hydrolysis of the ester was determined by titration with 0.01 *N* fixed alkaline hydroxide, using phenolphthalein as an indicator. Enzymic cleavage of the ester was not detected, hence an esterase was not present.

Jenny Hempel (15) found that "the sap of the stimulated pitcher of *Nepenthes* gives values for the hydrogen ion concentration greater than 10^{-7}, but unstimulated pitchers give no definite value."

Shibata and Nagai (16) noted the presence of flavone in the leaves and the flowers of *Nepenthes phyllamphora* Willd. More flavone was present in *N. phyllamphora*, growing in the open on the island of Yap, than occurred in *N. Mastersiana*, growing under glass at Tokyo.

Pfeffer (17) has suggested that the insectivorous plants derive both nitrogen and phosphorus from their prey.

Nepenthes have found application in medicine. "The water abstracted from their leaf pitchers is an article of commerce in the East Indies. The leaves and root of *N. Boschiana*, Krthls. are especially employed, chiefly as an astringent."(18)

II

A STUDY OF THE PROTEASE OF THE PITCHER LIQUOR OF NEPENTHES

BY

Joseph Samuel Hepburn, A.M., M.S., Ph. D.

Broadly speaking, two hypotheses exist concerning the mechanism of the proteolytic digestion within the pitchers of *Nepenthes*. One view is that digestion results from the action of a protease, secreted by the pitchers. The other view is that digestion is due to bacterial action. A third factor to be considered is the autolysis of the tissues of the dead insects.

In the present study, the volume of the liquor secreted by a single pitcher was always so small, that liquor could not be obtained from the same pitcher both before and after stimulation. Very rarely indeed did two pitchers mature on the same plant at the same time, thereby permitting a comparative study of the liquor from both stimulated and nonstimulated pitchers of the same plant. Differences due to individual plants could not be entirely eliminated, but the problem was attacked by several methods for the study of proteolysis, and a number of experiments were made according to each method; the results obtained by all the methods lead to the same general conclusions.

Material for this research has been obtained from the following species and hybrids of *Nepenthes*, grown in the Nepenthes House of the University of Pennsylvania:—*ampullaria*, *atrosanguinea*, *Chelsonii*, *Claytonii*, *Dominii*, *Dyerinana*, *gracilis*, *Hamiltoniana*, *Henryana*, *Hookeriana*, *Mastersiana*, *mixta*, *Morganiana*, *paradisae*, *Rafflesiana pallida*, *rufescens*, *splendida*, *Wittei*.

Pitchers were always selected prior to opening. They were closely watched; and the mouth of each pitcher was closed with absorbent cotton as soon as the lid opened; the entrance of insects was thereby prevented; and possible contamination of the pitcher liquor by the tissue enzymes of the digested prey was entirely excluded.

When the liquor from non-stimulated pitchers was studied, it was used as soon as possible after the opening of the pitcher.

When liquor from stimulated pitchers was desired, recourse was had to mechanical stimulation by chemically inert substances. In some experiments, the glands of the pitcher were stroked repeatedly with a camel's hair brush, and the cotton plug was then inserted; the liquor was removed for study on the following day. In other experiments, several small, round, solid glass beads, such as are used in fractionating columns, were inserted into the newly opened pitcher; the cotton plug was introduced; and the pitcher and its contents were shaken thoroughly at intervals during one or more days, taking care not to wet the cotton and thereby lose liquor; the liquor was finally removed for study.

In *all* the tests for the presence of a protease, a bactericide was used in order to exclude completely the action of micro-organisms. In some experiments, sufficient solid sodium fluoride was added to the mixture of pitcher liquor and substrate to render the final concentration of the fluoride 1 percent. In other experiments, a sufficient volume of a concentrated (2 percent.) aqueous solution of trikresol was added to render the final concentration of that bactericide 0.2 percent. This concentration of trikresol was found satisfactory by Graves and Kober (19) in certain of their experiments with proteases. When the mixture of pitcher liquor and substrate was diluted to a definite volume, the concentrated trikresol solution was added before the dilution to the final volume was made.

In each experiment, a blank or control determination was carried out, using pitcher liquor which had previously been boiled, then permitted to cool to the temperature of the room; the control was made in exactly the same manner, in all other respects, as the determination proper. The control or blank was always compared with the determination proper, and due allowance was thus made for the possible action of any thermostable catalyst present in the pitcher liquor, and also for any action of the reagents on each other.

The following reactions for the detection of a protease were used:—

(1) The formol-titration of Sörensen.

(2) The digestion of:—
    (a) carmine fibrin,
    (b) edestan,
    (c) protean derived from castor bean globulin,
    (d) ricin (Jacoby).

(3) The cleavage of glycyltryptophane.

The temperature of incubation was 37°C., unless otherwise stated.

Formol-titration

The following substrates were used:—ovalbumen, fibrin, edestin, ovomucoid, Nährstoff-Heyden, and Witte peptone. The fibrin was prepared from ox blood; the ovomucoid was obtained by the procedure of Eddy (20); the Nährstoff-Heyden, according to Gotschlich (21), was a mixture of different albumoses.

After incubation, any insoluble protein was removed by filtration, and was washed on the filter; the combined filtrate and washings were made neutral to phenolphthalein. If metaprotein separated, it was filtered out and washed on the filter; the filtrate and washings were again made neutral to phenolphthalein—if necessary—and one-half of their volume of formol (40 percent. formaldehyde), previously rendered neutral to phenolphthalein, was added. The basic amino group in the amino acid molecule was thereby converted into its methylene derivative by condensation with the formaldehyde, and no longer neutralized the acidic carboxyl group in the same molecule. This carboxyl group now functioned as an acid, giving the solution a reaction acid to phenolphthalein. This acidity, due to amino acids, was immediately titrated with standard fixed alkaline hydroxide, using phenolphthalein as the indicator, and *served as a measure of the proteolysis.* Usually 0.1 N sodium hydroxide was used for the titrations; however, 0.05 N sodium hydroxide was used when it was expected that the proteolysis would be slight on account of the small volume of pitcher liquor used.

The following experiments were made with the liquor from *stimulated* pitchers.

Ovalbumen (0.05 gram) was digested with 15 cc. of pitcher liquor for 3 days. After the addition of formol, the determination proper required 0.15 cc., the blank experiment 0.00 c.c. 0.05 N sodium hydroxide for the neutralization of the amino acids.

Fibrin (0.05 gram) was digested with 15 cc. of pitcher liquor for 14 days; on titration after the addition of formol, the determination proper required 0.45 cc., the blank 0.00 cc 0.1 N sodium hydroxide.

Ovomucoid (0.05 gram) was dissolved in 10 cc. of water, then incubated with 5.5 cc. of pitcher liquor for 6 days. The formol titration was:- determination proper 0.10 cc., blank 0.00 cc. 0.1 N sodium hydroxide.

Nährstoff-Heyden (15 cc. of a 1 percent. aqueous solution) was mixed with 15 cc. of pitcher liquor, then incubated for 3 days. The formol titration was:- determination proper 0.30 cc., blank 0.10 cc. 0.1 *N* sodium hydroxide.

Witte peptone (25 cc. of a 1 percent. aqueous solution) was mixed with 25 cc. of pitcher liquor, then incubated for 4 days. The formol titration was:— determination proper 4.60 cc., blank 1.85 cc. 0.1 *N* sodium hydroxide.

A solution of edestan was prepared by dissolving 0.1 gram of edestin in 15 cc. of 0.1 *N* hydrochloric acid, previously diluted to 50 cc. with water. The pitcher liquor (8 cc.) was mixed with 25 cc. of this solution (equal to 0.05 gram of edestin), and then incubated for 25 days. The formol titration was:— determination proper 0.90 cc., blank 0.00 cc. 0.1 *N* sodium hydroxide.

When liquor from *non-stimulated* pitchers was used, the following results were obtained.

In three experiments, the period of incubation was 4 days. In the first experiment, 10 cc. of pitcher liquor and 0.05 gram of ovalbumen were used; in the second experiment, 12.5 cc. of pitcher liquor and 25 cc. of a 1 percent. aqueous solution of Nährstoff-Heyden; in the third experiment, 12.5 cc. of pitcher liquor and 25 cc. of a 1 percent aqueous solution of peptone (Witte). In all three experiments, the formol titration of the determination proper was the same as that of the blank, showing that enzymic cleavage of the substrates had not occurred.

A solution of edestan was prepared by dissolving 0.1 gram of edestin in 15 cc. of 0.1 *N* hydrochloric acid, previously diluted with water to a volume of 25 cc. In one experiment, 25 cc. of the edestan solution were mixed with 11.5 cc. of pitcher liquor; sufficient water was added to make a total volume of 50 cc., and the solution was digested for 28 days. In another experiment, 9 cc. of the edestan solution were added to 8 cc. of pitcher liquor; sufficient water was added to make a total volume of 25 cc., and the solution was incubated for 21 days. In both experiments, both the determination proper and the blank remained neutral, after neutral formol had been added in the formol titration, hence digestion with the production of soluble proteolytic products had not occurred.

Carmine Fibrin

The directions of Grutzner (22) for the use of this reagent were some-somewhat modified. The carmine fibrin was washed with water to remove the glycerol, in which it had been preserved, then was permitted to swell in 0.2 percent. hydrochloric acid, to which sufficient trikresol had been added to produce a 0.2 percent. solution of that bactericide. The swollen, gelatinous carmine fibrin was placed in the pitcher liquor; sufficient hydrochloric acid (0.6 percent. solution) and trikresol (2 percent. solution) were added to make a concentration of 0.2 percent. of each of these reagents in the resulting solution. *The temperature of incubation was that of the room.* The occurrence of digestion was made known by two phenomena— (1) the flocks of carmine fibrin decreased in size and finally dissolved completely; and (2) the carmine was thereby liberated, dissolved, and imparted a red color to the solution.

In the *preliminary* series of experiments, the carmine fibrin was swollen in one mass, and a definite volume of the gelatinous reagent was used in each experiment. Three experiments were made in each of which the liquor from a single, *stimulated* pitcher was used. In the first experiment, the pitcher liquor (1.5 cc.) completely dissolved 0.1 cc. of carmine fibrin in 13 hours. In each of the other experiments, 4 cc. of pitcher liquor were permitted to act on 0.5 cc. of carmine fibrin; in one of these experiments, the substrate was partially dissolved in 15 hours, and completely dissolved in 24 hours; in the other experiment, the substrate was markedly digested in 48 hours, and completely dissolved in 6 days. In still another experiment, 4.75 cc. of liquor, collected from several *stimulated* pitchers, completely dissolved 0.25 cc. of carmine fibrin in 26 hours.

Liquor (1.5 cc.) from a single *non-stimulated* pitcher completely dissolved 0.1 cc. of carmine fibrin in 13 hours. The liquor (4.75 cc.) from several *non-stimulated* pitchers produced marked digestion, but not complete solution, of 0.25 cc. of the same substrate in 31 hours.

In the *final* series of experiments, the liquor from a *separate* pitcher was used in each experiment, and the carmine fibrin (0.2 gram) for each experiment was weighed out into a separate tube.

One set of experiments was conducted on liquor from *stimulated* pitchers. The carmine fibrin for each experiment was swollen in its tube in the usual manner, then was placed in the pitcher liquor, and hydrochloric acid and trikresol were added as described above. The time required to dissolve the swollen substrate was:—

Pitcher A, 3.5 cc. liquor, 48 hours.
" B, 2.5 cc. " 72 "
" C, 2 cc. " 93 "
" D, 3.5 cc. " 111 "
" E, 1 cc. " 133 "

Another set of experiments was made with liquor from *non-stimulated* pitchers; *unswollen* carmine fibrin was used, and *no acid* was added to the reaction-mixture. The pitcher liquor was placed on the substrate, and sufficient trikresol (2 percent. solution) was added to give a concentration of 0.2 percent. of the bactericide. Six experiments were made, the volumes of pitcher liquor being 1.0, 1.5, 2.5, 3.0, 3.5 and 5.5 cc. respectively. Even after 70 days, the substrate was absolutely unattacked. The supernatent liquid had assumed a very faint pink tinge, no more marked than that of a blank experiment.

In a third set of experiments, unswollen carmine fibrin was incubated with liquor from *non-stimulated* pitchers, in the presence of both hydrochloric acid and trikresol, as described above. Two experiments were made, the volumes of pitcher liquor being 2.5 and 1.0 cc. respectively. The substrate was markedly digested, in the first experiment in 16 hours, in the second experiment in 52 hours.

Edestan

The solution of edestan, used in these experiments, was prepared by dissolving 0.1 gram of edestin in 15 cc. of 0.1 *N* hydrochloric acid, previously diluted to 25 cc. with water. After the mixture of edestan solution and pitcher liquor had been incubated under the conditions stated below, it was neutralized with 0.1 *N* sodium hydroxide, using phenolphthalein as the indicator.

Liquor from *stimulated* pitchers was used in the following experiments:

The pitcher liquor (20 cc.) was mixed with 25 cc. of edestan solution; the mixture was diluted to a volume of 50 cc. with water, and was incubated for 14 days. Then the solution was neutralized. The determination proper gave absolutely no precipitate, showing that proteolysis had occurred, and that both the protean, edestan, and the meta-protein, which is one of the first products of proteolysis, had been converted into simpler proteolytic products. In the blank, on the other hand, a voluminous precipitate formed.

In a second experiment, 1 cc. of pitcher liquor and 2 cc. of the edestan solution were mixed; and the mixture was diluted with water to a volume of 5 cc., then incubated for 8 days. On neutralization, the determination proper failed to give a precipitate, while the blank yielded a voluminous precipitate. Hence the edestan had been digested.

Experiments were also made, using the liquor from *non-stimulated* pitchers:—

In one experiment, 1 cc. of liquor, 2 cc. of the edestan solution, and sufficient water to render the total volume 5 cc., were mixed; and the mixture was incubated for 8 days. On neutraliztion, a voluminous precipitate formed in the blank; the precipitate, which formed in the determination proper, was about one-half as great as that in the blank, showing that partial digestion of the edestan had occurred.

In another experiment, a mixture of 4 cc. of pitcher liquor and 1 cc. of the edestan solution was incubated for 13 days. On neutralization, the blank yielded a voluminous precipitate, the determination proper, a precipitate but one-tenth as great as that in the blank. Hence partial digestion of the edestan had occurred.

Protean derived from castor-bean globulin

The castor-bean globulin, used in these experiments, was presented by Dr. Isaac F. Harris, to whom I am also indebted for the outline of its preparation. Ground castor-beans were extracted with gasoline to remove the oil, then were extracted with a 10 percent. sodium chloride solution. This solution was filtered, and the clear filtrate was dialyzed. The globulin, which deposited, was dissolved in a 10 percent. solution of sodium chloride; the solution was filtered and dialyzed. The globulin, which separated, was washed with water, alcohol, and ether, and was desiccated.

A 2 percent. solution of this globulin in a 5 percent. solution of sodium chloride was used as a reagent for proteolytic enzymes; the solution was filtered, if necessary. When the clear solution of the globulin was mixed with the pitcher liquor and 0.5 cc. of 0.1 *N* hydrochloric acid was added, a cloudy precipitate of the protean derived from the globulin formed. On incubation, if a proteolytic enzyme, active in the presence of hydrochloric acid, was present, the insoluble protean was digested and converted into less complex, soluble compounds, which dissolved; and the cloud gradually became less dense, and finally disappeared.

The following experiments were made on liquor from *stimulated* pitchers.

The pitcher liquor (2.5 cc.) was incubated with 2 cc. of the globulin solution and 0.5 cc. 0.1 *N* hydrochloric acid. Proteolysis was marked on the third day, advanced on the seventh day, and almost complete on the twelfth day.

The experiment was repeated using 0.5 cc. of pitcher liquor, 4 cc. of the globulin solution, 1 cc. 0.1 *N* hydrochloric acid, and 4.5 cc. of water (to secure the same concentration of substrate and of acid as in the preceding experiment). The proteolysis was marked on the fourth day, and was almost complete on the ninth day.

Liquor from *non-stimulated* pitchers was used in all of the following experiments.

The liquor (0.6 cc.) was digested with 2 cc. of the globulin solution 0.5 cc. 0.1 *N* hydrochloric acid, and 1.9 cc. of water. No proteolysis had occurred at the end of 14 days.

In another experiment, 2.5 cc. of the liquor, 2 cc. of the substrate solution, and 0.5 cc. 0.1 *N* hydrochloric acid were mixed and incubated; the protean was completely digested in 14 hours.

Two experiments were made, using 1 cc. of liquor, 2 cc. of the substrate solution, 0.5 cc. 0.1 *N* hydrochloric acid, and 1 cc. of water. The protean was almost completely digested in one of these experiments in 29 hours, and was completely digested in the other experiment in 48 hours.

It should be noted that liquor from a separate pitcher was used in each experiment in which this protean served as the substrate.

Jacoby's Ricin

This test was carried out with the reagents prescribed by Jacoby (23). A solution of 1 gram of ricin (Jacoby) and 1.5 grams of sodium chloride in 100 cc. of water was prepared, and filtered if necessary. The pitcher liquor (1 cc.) and the ricin solution (3 cc.) were mixed; 1 cc. of 0.56 percent. hydrochloric acid was added; and the resulting mixture was incubated. In the presence of a protease active in this concentration of hydrochloric acid, the cloudy precipitate, which forms on the addition of the acid, is dissolved during subsequent incubation. The reactions involved are essentially the same as those described above for the castor-bean globulin.

Liquor from a *stimulated* pitcher was used in one experiment. The cloudy precipitate underwent a marked proteolysis in two days.

Liquor from a *non-stimulated* pitcher was used in another experiment. The cloudy precipitate was partially digested in two days, but had not been entirely digested at the end of 1 week.

Glycyltryptophane

Liquor (10 cc.) from *stimulated* pitchers was incubated with 2 cc. of an aqueous solution of the dipeptide, glycyltryptophane (so-called Ferment diagnosticum). In this series of experiments only, toluene was used as a bactericide. After incubation, the test for free tryptophane was made in the usual way with dilute acetic acid and bromine vapor; the production of a red color showed the presence of free tryptophane, and cleavage of the dipeptide.

In the first experiment, the period of digestion was nine days in the incubator; the test for free tryptophane was negative.

In a second experiment, the period of digestion was 21 days in the incubator, followed by 7 days in the room. A distinctly positive test for free tryptophane was obtained.

General Summary

The following conclusions may be drawn from the experiments reported.

The *formol titration* showed that the liquor from stimulated pitchers produced proteolysis of ovalbumen, fibrin, ovomucoid, Nährstoff-Heyden, and Witte peptone, while the liquor from non-stimulated pitchers lacked proteolytic power. This method also showed that, in the presence of very dilute hydrochloric acid, edestan was digested by the liquor from stimulated pitchers, but not by that from non-stimulated pitchers.

Carmine fibrin was dissolved by the liquor from both stimulated and non-stimulated pitchers, in the presence of 0.2 percent. hydrochloric acid. This substrate was not dissolved by liquor from non-stimulated pitchers in the absence of acid.

In the presence of very dilute hydrochloric acid, the pitcher liquor produced proteolysis of *edestan*; digestion proceeded more rapidly in liquor from stimulated pitchers than in liquor from non-stimulated pitchers.

The *protean derived from the globulin of the castor bean* was usually dissolved by the liquor from both stimulated and non-stimulated pitchers, in the presence of very dilute hydrochloric acid. The same statement may be made concerning Jacoby's *ricin*.

The liquor from stimulated pitchers apparently hydrolyzed glycyl-tryptophane, provided the period of incubation was sufficiently long.

The liquor from *stimulated* pitchers possessed proteolytic power in both the absence and the presence of acid.

The liquor from *non-stimulated* pitchers exerted no proteolytic power in the absence of acid, but possessed such power in the presence of acid.

Further study is required to determine the manner in which stimulation imparted *active* proteolytic power to the pitcher liquor. Possibly stimulation gave rise to a change in the reaction (hydrogen ion concentration) of the liquor and thereby created a favorable environment for the activity of an enzyme already present; or stimulation may have produced the activation of a zymogen present in the liquor; or it may have increased the secretion of protease by the glands of the pitcher.

In the presence of acid, liquor from stimulated pitchers digested certain substrates more rapidly than did liquor from non-stimulated pitchers; this was especially true of edestan.

The proteolytic enzyme of the pitcher liquor undoubtedly plays a highly important rôle in the digestion of insects within the pitcher.

III

A Bacteriological Study of the Pitcher Liquor of Nepenthes

By

Joseph S. Hepburn, Ph. D. and E. Quintard St. John, M. D.

Since certain investigators (9-10) have attributed the digestive action of the pitcher liquor of *Nepenthes* to the activity of micro-organisms, it has seemed desirable to study the bacterial content of the pitcher liquor, and the proteolytic power of its bacteria.

Description of the Media

Bacterial counts were obtained by sowing the pitcher liquor—undiluted, and in several successive dilutions—on plain nutrient agar, incubating the plates at 37°C., and counting the colonies in the usual manner.

For the study of the proteolytic activity of the bacteria of the pitcher liquor, certain protein media were used, following, to a large extent, the directions of Crabill and Reed (24). The basis of these media was a stock solution which contained magnesium and ferrous sulphates, dipotassium phosphate, and potassium chloride. For solid media a stock agar was prepared by addition of 2 percent. of agar to this solution. The protein solid media were obtained by addition of approximately 1 percent. of *one* of the following proteins to the stock agar:—casein, egg albumen, carmine fibrin, edestin, ricin (Jacoby), *protein* (prepared from aleuronat). After these media had been sterilized, the proteins were present as suspended, insoluble particles. Whenever proteolytic bacteria were present in the pitcher liquor plated on such media, their colonies gradually digested and dissolved the suspended particles over which they grew.

In a few experiments, plates of plain nutrient gelatin were also sown with the pitcher liquor, in order to detect the presence of liquefying (proteolytic) micro-organisms.

To test for the liberation of tryptophane and the formation of indol, a liquid medium was prepared, containing 0.4 gram *protein* (from aleuronat), 20 cc. 0.1 *N* sodium hydroxide solution, and 80 cc. of the stock solution of inorganic salts already mentioned. The resulting suspension of protein was placed in tubes (10 cc. to a tube), and was sterilized. The protein gave a purple color with glyoxylic acid and sulphuric acid (reaction of Hopkins and Cole), and therefore contained a tryptophane group in its molecule.

The formation of basic compounds (e.g. ammonia) from simple organic compounds of nitrogen was also studied, using as substrates:—glycocoll (an amino acid), acetamide (an acid amide), asparagin (which is both an amino acid and an acid amide), and ammonium lactate (an ammonium salt of an organic acid). For this study, recourse was had to solid media, prepared by addition of *one* of the compounds just named to the stock agar. One percent. of asparagin was used, and the other compounds in molecular concentration equal to that of the asparagin. One-half percent., by volume, of a two percent. solution of rosolic acid in sixty percent. alcohol was added to each medium as an indicator. These media were always sterilized by the discontinuous method. The production of basic compounds by bacteria growing on these media was indicated by a red color of the medium beneath and surrounding the colony. Sterile plates of the rosolic acid media were always poured as controls, to be used in determining the changes in color in the experiments proper.

In inoculating all of the special media just described, 1 cc. of the undiluted pitcher liquor was sown into each plate or tube.

Lactose bile-salt broth was used to test for the presence of members of the colon-aerogenes group of bacteria.

The temperature of incubation was always 37°C., except for the gelatin plates which were kept at 20° C.

Sources of the Pitcher Liquor Examined

In the majority of the experiments, the liquor was obtained either from unopened pitchers or from active, open pitchers containing insect remains. A few experiments were conducted on liquor from pitchers partly opened and not yet invaded by insects. *The liquor from each pitcher was studied as a separate experiment.*

Unopened Pitchers

The prolonged midrib or tendril, which carries the pitcher, was severed at the end of the basal part of the lamina; and that portion of the tendril, which interfered with the subsequent manipulation, was removed. Sterile scissors were always used in cutting the plant tissues. The top portion of the pitcher was rapidly passed through the flame, and was then cut off. The cut edge of the pitcher was then rapidly flammed; and the liquor was immediately withdrawn by means of a sterile pipette, and plated in the usual way on plain nutrient agar. The liquor from 12 unopened pitchers was studied in this manner, and was invariably found to be *sterile*, as was shown by the absence of colonies on the plates after incubation for 4 days.

Opened Pitchers

The liquor was removed from opened pitchers with sterile pipettes, placed in sterile test tubes, and immediately plated.

Partly opened pitchers, free from insects, were used in two experiments. The liquor from each of these pitchers contained a goodly number of bacteria which grew on plain nutrient agar.

The remaining experiments were conducted on liquor from open, active pitchers, containing insect remains. The number of bacteria present in 1 cc. of liquor was determined in each of five pitchers with the following result:—

Pitcher 1	450,000
2	8,000,000
3	1,200,000
4	1,900,000
5	48,000

The morphology of these bacteria was studied. Smears were made from several colonies, which differed in physical appearance, and were stained with Loeffler's alkaline methylene blue. All the micro-organisms were rod-like, and therefore belonged to the family of the *Bacteriaceae*. A few of the organisms contained spores; none of them produced gas when inoculated into lactose bile-salt bouillon.

The liquor in an old pitcher, which was becoming brown at the top, contained 104,000 bacteria per cc.

Gelatin. In two experiments, gelatin plates were poured. The bacteria grew and completely liquefied the gelatin in 48 hours.

Action on Special Media

After the plates of the special media had been inoculated, they were held in an incubator at 37°C., and were examined at intervals as stated below, until drying of the media rendered further observation useless.

Casein agar. Eight experiments were made using this medium. In seven experiments, growth, but no proteolysis, had occurred at the end of 3 days; digestion of the casein had begun by the fifth day, had become more marked by the ninth day, and still more marked by the twelfth day. In the eighth experiment, bacterial colonies failed to develop.

Egg albumen agar. Two series of experiments were made with egg albumen agar as the substrate. The first series included eight experiments; colonies had appeared in three experiments by the third day and in a fourth experiment by the ninth day. The plates in the other experiments remained sterile. Digestion of the albumen was not noted, even at the end of 12 days.

In a second series, which consisted of seven experiments, growth of the bacteria occurred during the first five days of incubation, but proteolysis of the albumen had failed to develop at the end of 14 days. Possibly, in both series, sufficient ovomucoid (a non-coagulable protein) was present in the dried egg albumen to supply the bacteria with the necessary carbon and nitrogen.

Carmine fibrin agar. Six experiments were conducted on carmine fibrin agar. Washed, unswollen carmine fibrin had been used in the preparation of the medium, and the flocks were rather large. Colonies developed by the third day, and showed a marked tendency to grow over the flocks. Proteolysis had not become apparent on the fifth day. On the ninth day digestion of the fibrin was distinctly under way.

Edestin agar served as the medium in four experiments. Growth of the bacteria, and possibly incipient proteolysis of the edestin, occurred by the third day. No further change was noted on the fifth day. The digestion of the edestin had advanced somewhat by the ninth day, and was still more marked on the twelfth day.

Ricin agar was used as the medium in three experiments. Colonies developed in one experiment by the third day, and in another experiment by the fifth day. Digestion of the ricin had begun in both experiments by the ninth day, and had become very marked by the twelfth day. Bacterial growth failed to occur in the third experiment.

Protein agar. Agar, containing protein from aleuronat, served as the medium in eight experiments. On the third day colonies were present in all the experiments, and proteolysis had probably begun in six experiments. On the fifth day distinct evidences were noted of incipient digestion of the protein in all eight experiments; this digestion was more marked on the ninth day, and still more marked on the twelfth day.

Each pitcher did not always contain sufficient liquor to permit a complete set of experiments on all six of the agar media which contained suspended proteins. However, a general tendency existed that, if the micro-organisms present in the liquor grew on one of these media, they grew on all of the media, and usually exerted a proteolytic action on all the proteins.

Asparagin rosolic acid agar. This medium was used in three series of experiments. In the first series which included seven experiments, colonies had developed by the fifth day, and a red (alkaline) color had been imparted to the medium. By the fourteenth day, the medium had become yellow in color (acid in reaction).

The second series consisted of eight experiments. Colonies appeared on all the plates by the third day. The entire medium next became alkaline in reaction; this change had occurred on from the third to the fifth day. While the colonies themselves remained alkaline, the medium finally became acid in reaction; this change had taken place in over one-half of the experiments by the ninth day, and in the remaining experiments by the twelfth day.

The third series included seven experiments, in six of which good growth of the bacteria and an alkaline reaction of the medium were apparent by the third day. The medium had become acid in reaction in one of these experiments by the tenth day. In the seventh experiment of this series, growth had not occurred by the third day, but both colonies and the alkaline reaction of the medium had developed by the tenth day. The liquor from this series of seven pitchers was also sown on glycocoll rosolic acid agar, acetamide rosolic acid agar, and ammonium lactate rosolic acid agar; the results are given below.

Glycocoll rosolic acid agar. In six experiments, good growth of the bacteria had occurred, and an alkaline reaction had been imparted to the entire medium at the end of three days; in one of these experiments, about one-half the total area of the agar had become acid in reaction by the tenth day. In the seventh experiment, bacterial growth did not occur.

Acetamide rosolic acid agar. In six experiments, good growth of the bacteria and an alkaline reaction of the medium were noted by the third day. On the tenth day, the medium was still alkaline in three experiments, and had become distinctly acid in two experiments, while the change from an alkaline to an acid reaction was almost, but not entirely, complete in the sixth experiment.

In the seventh experiment, colonies were absent on the third day, but had developed by the tenth day, and had caused the entire medium to assume an alkaline reaction.

Ammonium lactate rosolic acid agar. Six experiments were characterized, on the third day, by good growth of the bacteria and an alkaline reaction of the medium. On the tenth day, the reaction of the medium had begun to change from alkaline to acid in five of these experiments.

In the seventh experiment, growth of the bacteria had not occurred by the third day, but had taken place by the tenth day, and the medium had then become alkaline in reaction.

In the set of seven experiments, which were studied on all four rosolic acid agars—asparagin, glycocoll, acetamide, and ammonium lactate—the odor was also recorded. The plates were quite frequently characterized on the third day by an odor recalling that of ammonia or amines. This odor was rarely noted on the tenth day.

Protein liquid medium. The bacteria of the pitcher liquor were permitted to act on the suspension of protein (from aleuronat) in the stock solution of inorganic salts. Two series, of 8 experiments each, were made. In each experiment, 1 cc. of pitcher liquor was added to a tube of the medium.

In the first series of experiments, the test for indol was made after incubation for three days, and again after incubation for ten days. The test was always negative.

In the second series of experiments, neither indol nor *free* tryptophane was present after incubation for twelve days.

Lactose bile-salt bouillon. In two experiments, 1 cc. of pitcher liquor was sown in lactose bile-salt bouillon; gas developed within 72 hours, showing the presence of organisms of the colon-aerogenes group in both pitchers.

The liquor from several pitchers was mixed, and five tubes of the bouillon were inoculated, using 1 cc. of the mixture, and of its 1:10, 1:100, 1:1,000, and 1:10,000 dilutions, respectively. Gas developed in all five tubes within 72 hours, hence it may be stated that at least 10,000 micro-organisms of the colon-aerogenes group were present in 1 cc. of the pitcher liquor examined.

General Summary

The following conclusions are based on the bacteriological experiments.

The liquor taken aseptically from unopened pitchers was found to be sterile.

The liquor in partly opened pitchers, which were free from insects, contained a goodly number of bacteria.

Liquor from open, active pitchers, containing insect remains, had a bacterial count of from 48,000 to 8,000,000 per cc. These organisms were rods (*Bacteriaceae*). The bacteria in the liquor from such pitchers liquefied gelatin, and grew on agar in which the sole source of nitrogen and carbon was either a protein (casein, egg albumen, carmine fibrin, edestin, Jacoby's ricin, protein from aleuronat), or a simple organic compound of nitrogen (glycocoll, acetamide, asparagin, ammonium lactate). The bacteria usually digested the protein in the medium, but the rate of digestion was exceedingly slow. The bacteria decomposed the simple organic compounds of nitrogen; an odor recalling that of ammonia and amines was frequently produced; the medium became alkaline in reaction; later on, this reaction changed to acid, but the bacterial colonies themselves remained alkaline. These bacteria did not liberate tryptophane nor produce indol in their action upon protein (from aleuronat). The pitcher liquor, on the average, contained at least 10,000 micro-organisms of the colon-aerogenes group per cc.

The following conclusions are supported by the results of our studies on the protease and the bacteria of the pitcher liquor.

The slowness, with which bacterial digestion of the protein occurred, shows that bacteria play but a secondary rôle in the digestion of the insects in the pitcher. The leading role in the digestion is played by the protease of the pitcher liquor.

The bacteria live in symbiosis with the *Nepenthes* plant, drawing their nutrition from the digested insects, and assisting somewhat in the digestion of the insects.

Needless to remark, the tissue enzymes of the insects may produce autolysis of their tissues, and thereby assist in the digestion.

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IV.

OCCURRENCE OF ANTIPROTEASES IN THE LARVAE OF THE *Sarcophaga* ASSOCIATES OF *Sarracenia flava*

By

Joseph Samuel Hepburn and Frank Morton Jones

The parasitic intestinal worms of man and the domestic animals contain antiproteases (antipepsin and antitrypsin), which effectively prevent the digestion of the parasite by the proteolytic enzymes in the digestive fluids of the host. This is especially true of *Ascaris* (1).

The pitcher liquor of *Sarracenia flava* contains a proteolytic enzyme (2). The larvae of certain species of *Sarcophaga* (*sarraceniae* Riley, *Rileyi* Aldrich, and *Jonesi* Aldrich) habitually occur in the pitchers of *Sarracenia flava*, where they are constantly bathed in the digestive liquor of the pitcher. This phenomenon suggested the examination of *Sarcophaga* larvae from *Sarracenia* pitchers for the presence of antiproteases. Live larvae obtained from open pitchers of *Sarracenia flava* were used in the study. Two series of experiments were made.

In the first series, 16 larvae (total weight 1.86 grams) were crushed, and ground with sand and 4.5 cc. of distilled water. The turbid solution and suspended tissue were removed by decantation, and were mixed with sufficient 95 percent alcohol to render the final concentration of the alcohol 60 percent. The precipitate which formed was collected on a filter and dried over calcium chloride in a dessicator. The filtrate was mixed with alcohol until the concentration of the latter was 85 percent; the precipitate which formed was negligible, although the antiproteases should have separated at this point.

The thought, that possibly the rather tough larval tissue had not been ground sufficiently to liberate the antiproteases, led to an examination of the first precipitate for these antienzymes. When thoroughly dry, the precipitate was separated from the filter paper, ground intimately with glass powder, and then triturated with 10 cc. of distilled water. A supernatant liquid was obtained by centrifugation; 2.5 cc. of this liquid and 2.5 cc. of a 1 percent solution of pepsin in 50 percent glycerol were mixed; and sufficient hydrochloric acid and trikresol were added to produce a concentration of 0.2 percent of each of these reagents. The resulting solution was allowed to stand for 2 hours at room temperature to permit the pepsin and the antipepsin (if present) to combine. A control experiment was made in which 2.5 cc. of physiological salt solution were substituted for the solution derived from the larvae. Carmine fibrin (0.2 gram, weighed, then swollen in 0.2 percent hydrochloric acid) was added to both the experiment proper and the control, and both were then incubated at room temperature. In the control, the carmine fibrin was completely dissolved in 1.75 hours. In the experiment proper, the carmine fibrin was not dissolved at the end of 12 days, but had been completely dissolved at the end of 17 days. Therefore antipepsin, an antiprotease, was present in the larvae, since the solution derived from the larvae markedly retarded the peptic digestion.

In the second series of experiments, 82 larvae (total weight 8.30 grams) were used. From the same gathering of larvae a number were bred to the adult fly, and proved, by examination of the male genitalia, to be *Sarcophaga sarraceniae* Riley, the first recognized *Sarcophaga* associate of *Sarracenia*. The larvae were ground with glass powder to an intimate mixture, which was thoroughly triturated with distilled water. The pasty mass was subjected to a pressure of 50 kilograms per square centimeter in a Buchner press; 48 cc. of press juice were obtained. The press juice was so cloudy that the edestan and casein tests could not be applied in the examination for antiproteases, and only carmine fibrin was used as a substrate.

Antipepsin. In the experiment proper, 12 cc. of press-juice and 12 cc. of a freshly prepared 0.2 percent aqueous solution of pepsin were mixed and allowed to stand at room temperature for 30 minutes to permit the pepsin and the antipepsin (if present) to combine. Sufficient hydrochloric acid (2 percent) and trikresol (2 percent aqueous solution) were then added to make the concentration of each 0.2 percent in the resulting solution; lastly, 0.2 gram of carmine fibrin was added. A control experiment was carried out in exactly the same manner as the experiment proper, save that 12 cc. of distilled water were substituted for the press-juice. The temperature of incubation was that of the room. In the control experiment, the carmine fibrin was completely dissolved in 45 minutes; in the experiment proper, it was partly dissolved in 14 hours and completely dissolved in 17 hours.

Antitrypsin. In the experiment proper, 12 cc. of press-juice and 12 cc. of a freshly prepared 0.2 percent aqueous solution of pancreatin (owing its proteolytic power to trypsin) were mixed, and held at room temperature for 30 minutes to permit the trypsin and the antitrypsin (if present) to combine. Sufficient 4 percent solution of sodium carbonate and 2 percent solution of trikresol were added to make 0.4 percent of the former and 0.2 percent of the latter reagent in the final solution; then 0.2 gram of carmine fibrin was added. A control experiment was made exactly like the experiment proper, except that 12 cc. of distilled water were substituted for the press juice. The incubation was made at room temperature. In the control experiment, the carmine fibrin showed signs of incipient digestion in 45 minutes, and had completely dissolved in 14 hours. In the experiment proper, the carmine fibrin was only partly dissolved at the end of 17 hours, but was completely dissolved at the end of 22 hours.

Since the press juice markedly retarded the digestion of carmine fibrin by both pepsin and trypsin, both antipepsin and antitrypsin were present in the larvae of *Sarcophaga sarraceniae.*

Thermo-stability of the antiproteases. The experiments proper were also carried out as described above, except that the 12 cc. portions of press juice were boiled and cooled to room temperature, then used without filtration. The protein in the press juice was coagulated by the heat on boiling. The digestion of carmine fibrin by both pepsin and trypsin was retarded to about the same extent as when unboiled press-juice was used. The coagulated protein of the press juice was dissolved completely by pepsin and by trypsin (pancreatin) only after digestion at room temperature for 7 to 8 days, the coagulum remaining long after the carmine fibrin had disappeared. These results indicate that the antiproteases—antipepsin and antitrypsin—of the larvae were thermostabile. They also indicate that the coagulated protein of the press-juice either adsorbed antiprotease and thereby resisted digestion, or else was in itself not readily digestible.

The methods used in the preceding experiments were based on those described by Fischer (1) and by Wohlgemuth (3) The trikresol served as a bactericide.

In this study, antiproteases have been found in the larvae of the *Sarcophaga* associates of the pitcher plant, *Sarracenia flava.* The larvae of other species of *Sarcophaga*, and of several other dipterous genera, are likewise able to live and escape digestion in an environment rich in proteolytic enzymes; probably these larvae also contain antiproteases which protect them from digestion. Thus *Sarcophaga haemorrhoidalis* Fall. can live in the human intestinal tract; Haseman (4) has recently published a detailed account of a series of cases of intestinal myiasis in man, due to the presence of the larvae of this species in the intestines; Aldrich (5) gives an additional and similar case in which the parasite was also positively identified as *S. haemorrhoidalis*, and cites other records where this species may have been the one concerned.

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